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Unfrozen water in apple shoots as related to their winter hardiness

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UNFROZEN WATER IN APPLE SHOOTS AS RELATED TO THEIR WINTER HARDINESS

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INTRODUCTION

In breeding apples for a region where winter hardiness is an important consideration, the desirability of having some rapid and reliable means of separating the winter hardy seedlings from more tender specimens is obvious. Any eliminations that can be made in the first year or two of seedling growth are economical from the standpoint of both time and cost.

A number of different methods and procedures have been employed in an attempt to separate cold resistant plants from those less resistant to freezing temperatures. A survey of the literature related to this problem suggests that the capacity of a plant to retain its moisture against the extracting forces of freezing is associated with its ability to survive cold. With this generalization in mind the present investigation was undertaken to ascertain whether winter hardiness in apple shoots could be related to the quantity of water remaining unfrozen at low temperatures.

The meaning of the term "bound water" as used in the literature is controversial and will, therefore, be avoided in this discussion. "Water retaining capacity" as used subsequently refers to the capacity of a tissue to retain water in the unfrozen state when subjected to certain freezing temperatures.

LITERATURE REVIEW

Good bibliographies and reviews of the general literature on the influence of freezing temperatures on plants may be found in Chandler (4), Harvey (16), Maximov (29), Newton (34) and Rosa (43). An excellent discussion of the literature more closely related to the problem of winter hardiness in apple shoots is given by Hildreth (18). Jones and Gortner (24) included in their paper a comprehensive summary of the work published on the effect of freezing on colloidal systems. Gortner (12) considered also the water relationships in colloidal and living systems. Recently he (11) explained the nature and the methods of estimating "bound water." Critical examinations of the methods of measuring and the meaning of the term "bound water" are discussed by Briggs (2), Grollman (13), Newton and Gortner (37) and Hill (19, 20).

The literature reviewed here is of more particular interest to the study presented in the following pages. Robinson (42) showed that winter hardiness of insects was related to their ability to retain water in the unfrozen state when subjected to freezing temperatures. By the heat-of-fusion method he found that tender species were unable to increase their capacity to retain water in the unfrozen state when subjected to a temperature of -20°C. In contrast to these, hardy species exhibited considerable increase in this capacity when hardened under the same conditions as the tender species. Using the dilatometric technique Sacharov (45) obtained results in agreement with Robinson, although different species of insects were studied.

From his investigations on the winter hardiness of wheat plants, Newton (34) concluded that the volume of press-juice obtainable from hardened leaves was inversely proportional to the hardiness of a variety. The quantity of hydrophilic colloids contained in the press-juice, as measured by the method of Newton and Gortner (37), was found to be directly proportional to hardiness. By means of the dilatometer, Novikov (39) showed that the winter wheats which were more resistant to cold contained the greater quantities of unfrozen water when subjected to a temperature of -5.75°C. The amount of unfrozen water increased with the duration of hardening in the resistant group but very little or no increase was found in the non-resistant varieties.

Lott (26), working with brambles during the dormant season, observed a direct correlation between hardiness and percentage of unfrozen water as measured with the dilatometer at -6°C. Weimer (59) concluded from his studies on alfalfa roots that unfrozen water at -5°C. was related to the degree of hardening, but it could not be used to differentiate between hardy and nonhardy varieties. An increase in the quantity of unfreezable water during the hardening process in cabbage was brought out by Rosa (43), who determined the frozen water at -3, -4, -5 and -6°C. with the dilatometer. He concluded that the rate of decrease in percentage of freezable water coincided with the observed rate of hardening.

By measuring the quantity of expressed sap, Meyer (31) was unable to obtain evidence of any significant increase in the amount of unexpressable water in pine leaf tissue in winter as compared with summer. With calorimetric measurements at -20°C. he found greater quantities of both unfrozen and frozen water per gram of dry matter in the leaves in the summer than in winter. He concluded that increase in "bound" water played no role in cold resistance of this species, since non-living colloidal systems exhibit a positive correlation between total hydration and the amounts of both bound and free water per gram of dry matter.

In this paper Meyer suggested a lack of significance of previous results (30) in which he found the water retaining capacity of leaf tissue of the pitch pine greater in winter than during the summer.

MATERIAL AND METHOD

Material

The material used throughout this investigation consisted of shoots from 15 standard horticultural varieties of apple, *Malus sylvestris*. These varieties were selected so that degrees of hardiness from very tender to extremely hardy would be included. In descending order of their hardiness as based upon years of field observation at this station (27), the varieties may be arranged as follows: Hibernal, Virginia, Shield's Crab, Dudley, Okabena, Wealthy, Ioensis, Wolf River, Cortland, Baltimore, Jonathan, Delicious, Grimes, Wagner and Stayman.

Material subsequently referred to as "nursery shoots" consisted of shoots taken from stocks planted in 1924 and cut back to the crown each winter. Shoots of all varieties from this source averaged about 3 feet in length at the end of the growing season. The term "tree shoots" applies to tip growth of a single season from trees planted in 1926. These shoots were in general about 18 inches in length at the close of the growing season. Only 10 of the 15 varieties were available in tree form. Those not available were Hibernal, Shield's Crab, Okabena and Ioensis. The tree and nursery material was grown in adjacent plots and uniformity of soil and other conditions was considered in collecting samples.

Sampling

Shoots were collected before 9:00 a.m. on the day of determination and the leaves were immediately removed. After the shoots of a variety were weighed roughly to 60 grams, they were cut into three samples of approximately 20 grams each. Each sample was then quickly weighed on a torsion balance and transferred immediately to a stoppered test tube. As soon as the three samples of one variety were weighed, two of the tubes were placed in the cooling bath at -20°C. and the third in a refrigerator until used in either the heat or -5° treatment. Each sample weighed between 20.0 and 20.3 grams and consisted of pieces about 1 inch in length. Weighings of the fresh samples were accurate to 0.005 grams. In case of the tree shoots, two samples were cut instead of three.

The entire growth of the current year was used in sampling, except where a smaller portion was needed to bring the sample to the desired weight. In this instance a mid-section of another shoot supplied the deficiency. The importance of such care in sampling is discussed under a following section.

Method of Measuring Unfrozen Water

Although there are several errors involved in the application of the calorimetric or heat-of-fusion method to biological tissue, it still affords, with very little alteration of the material, the advantage of freezing conditions comparable to those in the field. This advantage is not found in the dilatometer method, which is more simple but was found to be less satisfactory for measuring frozen water in apple shoots. Varied attempts to apply the more simple dilatometric technique to this material resulted in repeated failure.

The application of calorimetry to biological tissue may be traced back to Muller-Thurgau (33) who estimated roughly the ice in plant tissue by dropping the frozen pieces of tissue into water and noting the resulting temperature change in the water. Later, Rubner (44) employed a similar procedure although somewhat more refined. Thoenes (56) is responsible for further modifications and improvements. Introduction of the method into American biological research should perhaps be credited to Robinson (42), who added still other refinements. With a few modifications the procedure used here is essentially that described by Thoenes (56) and Robinson (42).

Freezing the tissue

The tissue was frozen in test tubes, 25 x 250 mm., submerged in a calcium chloride brine solution that was cooled by a mechanical refrigerating unit. An insulated metal box holding about 52 liters of brine served as the cooling chamber. The brine was cooled continuously and heated to the proper temperature by two knife-type heaters which were attached to a super-sensitive dry cell thermo-regulator. This arrangement made possible a temperature control of within 0.1°C. when the solution was circulated by an electric mixer.

By placing the tubes immediately in the cold bath a very rapid drop in temperature occurred in the tube. When the brine was at -20°C., a period of 90 minutes elapsed before the temperature inside of the tube reached that of the bath, although -19.3° was reached in 40 minutes. This is a rather rapid freezing compared with usual field conditions.

Measuring unfrozen water

In brief, the heat-of-fusion procedure consists of measuring the change in temperature of a known quantity of water caused by the addition of the frozen material.

An ordinary wide-mouthed, pint thermos bottle was used as a container for the water. Two hundred ml. of water was first pipetted into the bottle. A large rubber stopper holding a stirring propeller and a thermometer was next seated in the mouth of the bottle. While the water in the calorimeter was being stirred, the temperature of the frozen sample was taken from a thermometer placed in one of the sample tubes. The temperature of the water in the thermos bottle was then read and the frozen sample was quickly poured from the test tube into the calorimeter with care to prevent excessive splashing to the walls. Stirring was resumed until the water reached a minimum temperature, when the second reading was made. After this final reading the sample was put in a weighing bottle for moisture determination. Moisture was calculated as the difference between the initial fresh weight and the weight of the sample after drying 60 hours at 80°C. under a vacuum of approximately one atmosphere.

Corrections for apparatus

Since the temperature of the walls of the bottle, the thermometer and the stirring propeller are changed along with that of the water, a correction is necessary to allow for this change in temperature of the apparatus. This correction is often referred to as the "heat capacity" or "water equivalent" of the apparatus. Also, the mid-point of the temperature change of the water in the bottle should be at room temperature or another correction is necessary.

These two corrections were made by following the same procedure described for measuring unfrozen water in the shoot samples, except that pure ice replaced the frozen tissue. Known quantities of water were frozen in small stoppered vials whose stoppers were removed one-half hour previous to use. Enough water was pipetted into the thermos bottle to make the height the same as when the frozen shoots were used. This procedure eliminated a correction for change in water level.

By making a series of determinations with the mid-point of temperature change in the water at, above and below room temperature, the correction for the variation from room temperature was found as well as for the water equivalent of the apparatus. In making the calculations (52) the heat-of-fusion of water was taken as 80 calories.

With the data obtained thus far, plus the specific heat of the tissue, it is possible to calculate the frozen water in a sample. From a number of determinations it was found that the specific heat at room temperature was a linear function of the percentage of water in fresh tissue (51). Consequently values for the specific heat were taken from a line constructed from these observations. This line is reproduced in fig. 1.

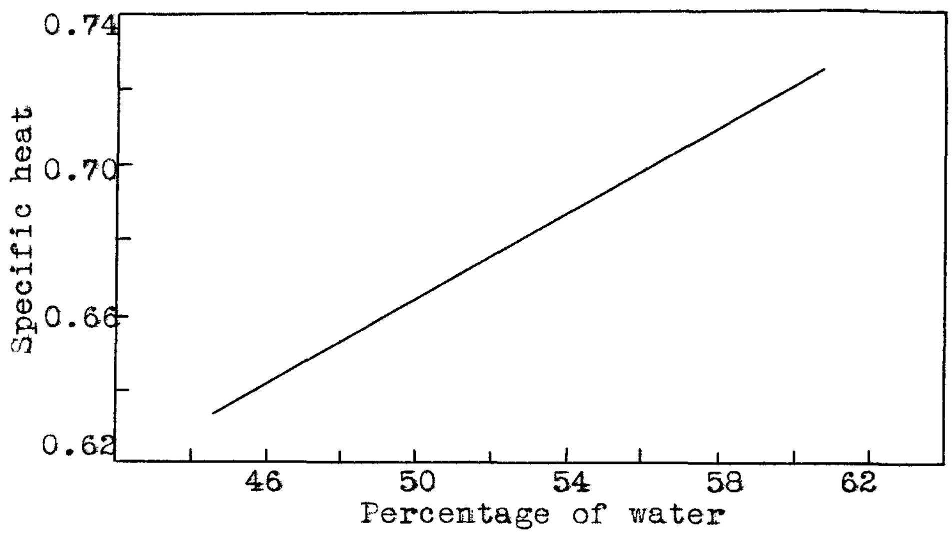


Fig. 1. Relation of specific heat to the percentage of water in fresh tissue.

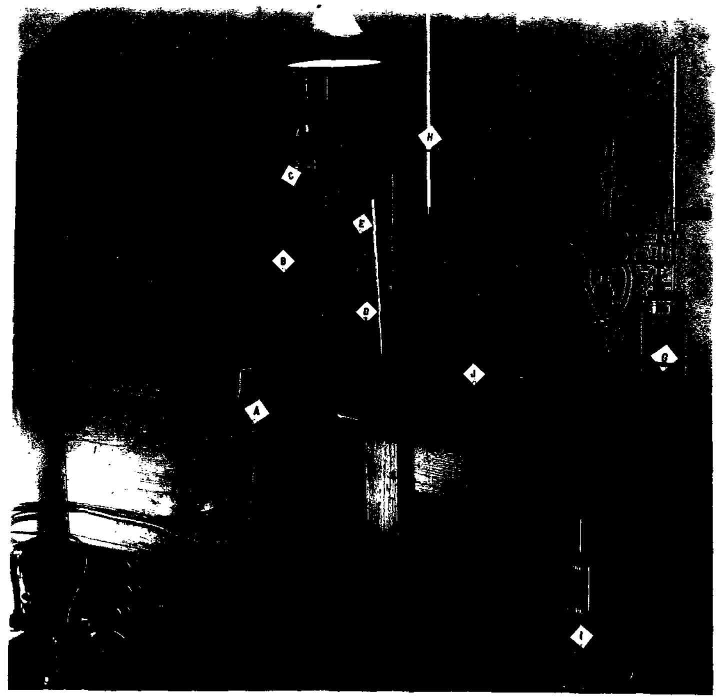
The frozen water in the sample may now be calculated by applying the data in the following formula, found in Thoenes (56) and slightly modified by Robinson (42).

$$x = \frac{nf\ (t_3 - t_2) - sw\ (t_1 + t_2)}{80 - 0.5t_1}$$

In which:
- x = frozen water in grams
- n = ml. of water in thermos bottle
- f = factor for apparatus
- t_1 = temperature of frozen tissue in degrees C.
- t_3 = initial temperature of water in bottle
- t_2 = final temperature of water in bottle
- s = specific heat of fresh material
- w = weight of fresh material in grams
- 80 = heat of fusion of water
- 0.5 = specific heat of ice

The absolute value for frozen water as calculated by this formula is not exactly correct. In the first place, no consideration is given to changes in the specific heat of the material with temperature. Also, the changing melting point of the frozen solution is neglected since this value is taken as $0^{o}C.$ for the entire solution. And further, in a solution of plant sap the heat-of-fusion is probably not that of pure water (79.7) but considerably less, perhaps nearer that of sea water (54.0), (49).

Other criticisms of the method are mentioned by Hill (20), St. Johns (47) and Meyer (31). In spite of the errors involved, an approximation of the frozen water is possible and the values obtained are at least comparable in the same type of material.



Photograph of apparatus. A, tubes joining coil and compressor; B, wire from relay to heating unit; C, relay; D, mercury thermo-regulator; E, motor to stir bath; G, thermos bottle; H, thermometer for room temperature readings; I, thermos jug; J, suction tube for pipette filling.

EXPERIMENTAL

Variation in the Material

There are several sources of variation in the material that must be considered in sampling. In shoots of the same variety there is likely to occur rather large differences in the water relationships of duplicate samples if the relative amounts of base and tip portions composing the two samples are not the same. This fact is brought out in table 1, where data are given for the tip and base portions of the same shoots.

Table 1. Water relationships in base and tip portions of the same shoots. Jan. 9, 1932.

Variety	Percentage of water			Percentage of water unfrozen		
	Tips	Bases	Diff.	Tips	Bases	Diff.
Hibernal	49.0	46.9	2.1	41.1	46.2	5.1
Virginia	51.4	47.6	3.8	38.2	44.3	6.1
Shield's	53.0	49.6	3.4	37.1	40.4	3.3
Dudley	51.0	48.3	2.7	39.7	44.5	4.8
Wealthy	50.4	47.0	3.4	39.1	45.0	5.9
Ioensis	51.7	47.5	4.2	39.8	45.6	5.8
Delicious	51.4	48.1	3.3	38.0	44.3	6.3
Mean	51.1	47.9	3.3	39.0	44.3	5.3

There is exhibited by the bases in every case a higher water retaining capacity against freezing than in the corresponding tips of the same shoots. A larger mean of 5.3 per cent for water unfrozen in the bases points to the relationship of unfrozen water to winter hardiness, since it is generally known that the tips of shoots are often killed during a winter that injures the bases very little.

From the results in table 1, the conclusion might be drawn that small differences in a comparison of varieties were entirely the result of varying quantities of base and tip tissue making up the samples. To test the validity of such a conclusion unfrozen water measurements were made on portions of shoots of the same diameter. Representative data from these determinations are found in table 2.

Table 2. Water relationships in shoots of the same diameter (3/16" to 4/16"). Jan. 18, 1933.

Variety	Percentage of water	Percentage of water unfrozen
Hibernal	47.0	44.1
Virginia	48.9	41.5
Dudley	46.6	45.6
Wealthy	49.0	42.0
Ioensis	48.6	42.3
Delicious	48.3	42.4
Stayman	46.5	44.6

It is evident from the data (Table 2) that there are measurable differences between varieties when sections of shoots with the same diameters are compared. Even though the variation in tip and base portions may account for some differences in a comparison of varieties, there are also other sources of difference to be considered.

Perhaps the greatest source of variability in water relationships within a single variety is the range of its individual shoots. In table 3 may be seen the total range in 15 individual shoots which were selected especially to demonstrate this point. The largest and smallest shoots in each of the two varieties were included in this selection. In the same table are data from the entire 15 varieties measured the preceding day.

Table 3. Range of 15 individual shoots in two varieties, and in the 15 varieties. May 20, 1933.

Variety	Percentage of water				Percentage of water unfrozen			
	High	Mean	Low	Range	High	Mean	Low	Range
Jonathan	57.5	55.2	53.8	3.7	31.0	28.0	25.6	5.4
Delicious	61.4	58.3	56.6	4.8	25.7	24.1	19.9	5.8
All varieties	58.8	55.5	53.0	5.8	30.6	27.2	24.2	6.4

It is apparent from the ranges in unfrozen water that shoots of the same variety behave very differently in this respect. Any sample collected on the day of this determination would probably fall within or near the values bounding the outside limits of the variety. The limits for the individual varieties are exceeded only very slightly by the range of the entire 15 varieties. Thus it would seem that under such a condition a comparison of the individual varieties would be valueless. It should be noted, however, that the means of the two varieties are not the same, and a later statistical analysis brings out the significance of this fact.

From the results discussed thus far a question might arise as to the possibility of obtaining satisfactory duplication in samples when several entire shoots were used in sampling. Table 4 yields light on this question as well as furnishing some information as to the accuracy of measurement of the unfrozen water.

Table 4. Water relationships in duplicate samples. Jan. 30, 1932.

Variety	Percentage of water			Percentage of water unfrozen		
	I	II	diff.	I	II	diff.
Virginia	48.4	48.2	0.2	41.2	42.8	1.6
Wealthy	49.0	49.1	0.1	41.5	41.4	0.1
Ioensis	49.0	49.4	0.4	43.2	43.0	0.2
Cortland	50.2	50.2	0.0	41.5	40.5	1.0
Jonathan	49.1	48.9	0.2	41.2	41.5	0.3
Delicious	51.1	51.0	0.1	38.4	38.9	0.5
Stayman	49.0	49.2	0.2	41.7	41.1	0.6
Mean	49.4	49.4	0.17	41.2	41.3	0.61

A comparison of the percentages of water in sample I and sample II indicates a rather close similarity in the duplicate samples, while the columns representing the percentages of water unfrozen are indicative of the accuracy of measurement. The larger differences in percentage of unfrozen water might be attributed to the greater possibilities for error involved in measuring this fraction. The data in table 4 are hardly representative of the variation in sampling, with a mean difference of only 0.61 in percentage of unfrozen water, since the mean difference in all determinations, including both duplicate and triplicate sampling, was 0.80 with one-half of the observations falling between 0.4 and 1.2. This error of measurement is mentioned again in the statistical analysis of the individual varieties.

The Water Relationships in Apple Shoots Under Various Treatments

A number of experiments were conducted to study the behavior of the substances responsible for holding water unfrozen as well as to further test the hypothesis that the capacity to retain water against freezing is related to winter hardiness. The following discussion is an account of these investigations.

Effect of slow and rapid freezing on unfrozen water

For some time there has prevailed among investigators a controversy over the influence of slow and rapid temperature fall in causing injury through freezing. Some plant investigators have observed more injury from a rapid drop in temperature than from a more gradual lowering. Others have been unable to substantiate this view. Hildreth (18) concluded that apple shoots frozen more slowly were injured less than those frozen more rapidly. Carrick (3) noted a similar response in apple roots. In an extensive study of the effect of rate of cooling, Chandler (4) showed that a rapid temperature drop caused more killing in fruit tree shoots than a corresponding slow cooling. Much additional evidence might be presented to show that rate of freezing is a factor in deciding the amount of injury.

In non-living systems Stiles (54) and Moran (32) have noted a marked difference in the manner and type of crystal formation with rate of cooling; a rapid cooling caused more numerous and smaller crystals to form. Moran (32) also observed that rapid and slow rates of freezing affected the structure of gels differently. This observation was confirmed by Hardy (14).

If rapid freezing causes greater injury than a corresponding slow freezing, it might be expected that more water would form as ice with a more abrupt temperature lowering. To determine the influence of rate of temperature fall upon the percentage of water frozen, a set of samples was placed in the cooling brine at 0° and allowed to cool to -20° in 12 hours.

When the temperature of -20° was reached the duplicate samples were added and freezing was continued at this temperature for 12 hours. In table 5 may be found representative data from this procedure.

Table 5. Unfrozen water in shoots frozen slowly and rapidly. Jan. 12, 1933.

Variety	Percentage of water			Percentage of water unfrozen		
	Slow	Rapid	Diff.	Slow	Rapid	Diff.
Hibernal	47.3	47.4	+0.1	45.3	45.6	+0.3
Virginia	48.9	49.0	+0.1	43.5	43.5	0.0
Dudley	47.5	47.5	0.0	47.5	46.2	-1.3
Wealthy	49.6	49.3	-0.3	41.9	42.9	+1.0
Ioensis	49.5	50.1	+0.6	43.8	43.7	-0.1
Delicious	48.5	47.9	-0.6	44.4	45.7	+1.3
Stayman	47.4	46.1	-1.3	46.2	47.6	+1.4
Mean	48.4	48.2	-0.2	44.6	45.0	+0.4

Any differences observable are very similar in size to those expected in duplicate samples which have been treated alike. Rapid cooling, then, did not decrease the quantity of water unfrozen over that found with a more gradual lowering of temperature.

It should be pointed out that slow cooling is not necessarily slow freezing since an undercooling of several degrees might occur. Johnston (23) observed incipient ice formation in peach buds at temperatures from -5.9° to -8.0°C. In the present investigation preliminary studies with the dilatometer showed that solidification began about 4 to 6 degrees below zero in apple shoots. On the basis of these crystallization temperatures the importance of undercooling is questionable.

Apparently the rapid rate of freezing employed here had no measurable influence on the quantity of ice formed in the tissue. It is certain that at this temperature no injury to the shoots was caused by either treatment and it may be that differences resulting from rate of cooling could be measured only at killing temperatures.

Effect of period of freezing on unfrozen water

In the killing of plant tissue by cold the influence of freezing time has been shown to be an important consideration. By prolonging the time from 3 to 12 hours, Hildreth (18) obtained an increase in the amount of injury in apple shoots at the same temperature (-43°C. on Jan. 2). Potter's (40) results with apple roots were in agreement with those of Hildreth. Newton and Brown (36) noted increased precipitation of proteins in expressed plant juices after longer exposures at the freezing temperature. If increased injury in apple shoots is caused by greater water loss through ice formation, it might be expected that more ice should form with longer exposures to the freezing temperature.

Experiments to test the influence of freezing time on the percentage of water unfrozen were conducted during the season of least hardiness as well as that of greatest cold resistance in the shoots. The results of these experiments are tabulated in table 6.

By comparing the values for unfrozen water in table 6a it may be seen that on May 23 a freezing period of 4 hours was long enough to establish a water-ice equilibrium in the tissue. On Jan. 18, however, equilibrium was not reached during the first 4 hours of freezing. This fact is evident from the smaller quantity of unfrozen water in the 24-hour period. The margin between the two periods of exposure is not large, but a statistical analysis of the data gave a highly significant difference between the values for 4 and 24 hours on Jan. 18. In the test for significance the value for "t"* was 9.77, while the highly significant value for "t" is only 2.97 (10).

Even though the water-ice equilibrium was not completed during the first 4 hours of freezing at -20° on Jan. 18, the data in table 6b show that equilibrium was reached in 24 hours on Jan. 4. The lack of a significant difference between 24 and 96 hours in unfrozen water on Jan. 4 indicates that all of the water that could be frozen out in 96 hours formed as ice in the first 24 hours of freezing.

* "t" is a value used in the test for significance of differences between means. See Fisher (10).

Table 6a and b. Influence of period of exposure to cold on unfrozen water.

a.

Variety	-20°							
	May 23, 1933				Jan. 18, 1933			
	4 hours		24 hours		4 hours		24 hours	
	Percentage of				Percentage of			
	water	unf.w.	water	unf.w.	water	unf.w.	water	unf.w.
Hibernal	57.1	25.9	57.8	24.7	47.3	45.2	47.5	42.4
Virginia	61.4	21.6	61.5	21.2	49.8	41.7	49.4	37.8
Dudley	53.4	29.4	53.3	29.0	48.8	43.4	47.8	41.1
Wealthy	56.7	25.6	57.8	24.8	49.2	43.8	48.1	40.3
Ioensis	58.2	23.8	59.1	22.8	50.6	42.0	49.8	40.0
Delicious	58.5	24.3	58.0	22.8	48.6	43.9	48.1	41.8
Stayman	54.3	29.6	54.6	28.7	48.3	44.3	46.9	41.3
Mean	57.1	25.7	57.4	24.9	48.9	43.5	48.2	40.7

b.

Variety	-20°				-5°			
	Jan. 4, 1933				March 1, 1933			
	24 hours		96 hours		4 hours		24 hours	
	Percentage of				Percentage of			
	water	unf.w.	water	unf.w.	water	unf.w.	water	unf.w.
Hibernal	46.2	44.9	46.2	44.1	46.9	59.5	47.6	60.5
Virginia	48.3	42.9	48.3	41.5	48.1	53.0	48.6	53.6
Dudley	47.7	43.8	47.7	43.8	47.7	55.0	48.5	54.6
Wealthy	49.6	42.3	49.6	39.4	47.8	55.8	48.4	55.4
Ioensis	47.5	43.1	47.5	43.1	49.5	53.7	50.3	53.7
Delicious	47.1	43.4	47.1	42.7	47.4	57.1	48.6	57.1
Stayman	45.5	42.9	45.5	43.0	45.3	62.8	46.0	62.5
Mean	47.4	43.3	47.4	42.5	47.5	56.7	48.3	56.8

Evidently the time required for the attainment of a water-ice equilibrium at -20° varied with the condition of the tissue. When the shoots were in the hardened state more time was necessary for the establishment of a water-ice equilibrium than when it was more readily killed by cold. Perhaps the longer freezing time required to reach equilibrium in the shoots while in the hardened condition is a factor in their surviving cold. The influence of time is important in a study of heat treatments on colloidal and living systems and it is possible that death from cold is a time-temperature relationship similar to death from heat.

The percentage of moisture in the tissue may be a factor in determining the time for attainment of equilibrium. Working with gels, Jones and Gortner (24) found that the time to reach equilibrium varied with the concentration and the temperature of exposure. At temperatures near the freezing point the rate of reaching equilibrium was slower than at lower temperatures. Gels of higher concentration froze more slowly than those with less dispersed material.

The data in table 6 are partly in agreement with these observations in gel systems. On May 23 the percentage of moisture in the tissue was high and equilibrium was attained in a shorter time than on Jan. 18, when the percentage of water was lower. This behavior is similar to that in the gel systems. On the other hand, the attainment of equilibrium in apple shoots at the temperature near the freezing point (-5°) was faster than at the lower temperature (-20°). The completion of an equilibrium in 4 hours at -5° is shown from a comparison of the unfrozen water in 4 and 24 hours on March 1. A glance at the water relationships in fig. 11, page 55, will show that the tissue was still in the hardened condition at this time and therefore a comparison with the measurements made on Jan. 18 is justifiable. The more rapid rate of reaching equilibrium at the lower temperature in apple shoots is just the reverse of that in gels as reported by Jones and Gortner (24).

<u>Effect of preceding low temperature on unfrozen water</u>

It would be valuable to know if exposure to cold brought about any changes in the substances retaining water in the unfrozen condition. To test the influence of low temperature on the subsequent behavior of these substances, two sets of samples were first frozen 4 hours at -20° and then warmed to -5°. When the latter temperature was reached, one set of samples was taken from the -5° bath and placed in water at room temperature for one hour. At the end of the hour this set of tubes was returned to the -5° bath along with a triplicate sample not yet frozen. All three sets, i.e., -20° not thawed, -20° thawed and -5° only, were then frozen 14 hours at -5°. The data obtained from this procedure are presented in table 7.

Table 7. Influence of preceding low temperatures on unfrozen water. May 3, 1933.

Variety	-5° only		-20° thawed		-20° not thawed	
	Percentage of					
	water	unf.w.	water	unf.w.	water	unf.w.
Hibernal	53.4	47.6	53.1	43.1	53.0	37.7
Virginia	55.0	44.0	55.3	39.6	55.3	30.8
Dudley	53.0	46.5	52.6	42.3	52.4	35.2
Wealthy	52.0	49.0	51.5	43.6	51.2	38.2
Ioensis	51.9	49.2	51.6	41.9	52.2	36.2
Delicious	53.3	46.5	52.7	43.0	53.2	33.5
Stayman	51.3	45.3	51.0	38.6	51.4	38.9
Mean	52.8	46.9	52.6	41.7	52.7	35.8

The triplicate samples of each variety (Table 7) appear to be quite similar if the percentages of water are any indication of their likeness.

By comparing the means for unfrozen water in the -5° only and the -20° thawed treatment it is clear that after complete thawing from the colder temperature a recooling to -5° froze out more water than was removed by -5° in the untreated sample. The greater quantity of water unfrozen in the -5° only treatment is evidence of the irreversibility of the process of freezing out and reabsorption of water in the tissue. A difference in unfrozen water of 5.2 per cent resulted even though the samples were allowed 20 hours in which to come to equilibrium after the initial freezing at -20°, one hour of which was at room temperature. It is probably correct to assume, from this decreased water retaining capacity of the thawed samples, that the lower temperature brought about some alteration in the organization of the substances responsible for the retention of water against freezing.

A comparison of the percentage of water unfrozen at -20° thawed and -20° not thawed (Table 7) will reveal the fact that part of the water originally frozen was reabsorbed by the thawed sample and was not removed by the subsequent freezing at -5°. This relationship is shown clearly by the difference in the means for unfrozen water. The thawed samples have a mean of 41.7, as compared with 35.8 for the samples not thawed. If all of the water originally frozen at -20° had been formed again as ice in the refreezing at -5°, it might be expected that the two means for unfrozen water would be the same. Since the thawed samples have a mean of 5.9 per cent larger than the samples not thawed, it may be concluded that this value represents the quantity of water reabsorbed and not frozen again at the temperature of -5°. When this value (5.9) is compared with 5.2 per cent, the difference between means of the samples held at -5° only and those held at -20° and thawed, it is seen that approximately one-half of the total water originally frozen at -20° remained in the liquid state at -5°. This is evidence that the freezing process is only partially reversible. If the process were completely reversible no difference in the means of the thawed and not-thawed samples would be expected. Jones and Gortner (24) were able to demonstrate complete reversibility in the gelatin systems they studied. In systems of inorganic hydrogels they found the quantity of water frozen to be increased by a previous lower temperature, thereby resembling apple shoots in their lack of reversibility. Thus the substances responsible for retaining water unfrozen in apple shoots behave more like inorganic or inelastic hydrogels than like gelatin, an elastic type of gel.

The data in table 7 are also of interest from the point of view of winter injury. If cold injury results from a dehydration of the protoplasm by the removal of water in the formation of ice, a period of severe cold followed by warmer weather that remained below freezing would probably be as injurious as if the temperatures had remained low. The larger percentage of water frozen in the -20° not-thawed treatment over that of the samples held at -5° only is evidence in favor of this suggestion. Thus it would seem that once the water has been removed as ice, it remains in the solid state even when the temperature is increased to a point where the water would not have frozen originally. This failure of the ice to melt indicates that colligative properties are of minor importance in retaining the water in the liquid state. If they were effective, melting of the ice could be expected at the warmer temperature.

Results similar to those discussed above were obtained with a temperature interval between -43° and -20°.

Effect of extreme cold on unfrozen water

A freezing temperature of -20°C. was used for the majority of determinations in this study principally because Rubner (44), Thoenes (56) and Robinson (42) have assumed that all of the "free water" and none of "bound water" is frozen at this point. Gortner (12) has stated that, "....when a part of the bound water is removed from the hydrophilic colloid, the colloidal structure is altered and vital function interfered with."

Since it appears from this that "bound water" is essential for the continuance of vital functions, it would be interesting to know how temperatures lower than -20°C. influenced the quantity of water remaining "bound" or unfrozen.

To determine this influence some observations were made on shoots frozen at -43° along with their duplicates at -20°C. Representative results of these observations are contained in table 8.

Table 8. Unfrozen water in shoots held at -43° and -20°. Jan. 2, 1933.

Variety	-43°		-20°		Order of hardiness
	Percentage of				
	water	unf.w.	water	unf.w.	
Hibernal	46.6	36.0	46.3	51.5	1
Virginia	48.0	32.9	47.8	46.4	2
Dudley	45.2	38.0	45.6	49.3	3
Wealthy	50.4	28.8	50.6	40.3	4
Ioensis	48.4	33.6	48.4	45.8	5
Delicious	48.2	33.3	48.8	45.6	6
Stayman	47.3	34.3	48.0	45.5	7
Mean	47.8	33.9	47.9	46.4	

From the data in table 8 it is clear that with a lowering of temperature there is an increased ice formation in the shoots. The samples held at -43° retained only 33.9 per cent of their water in the unfrozen state, as compared with a mean of 46.4 per cent in the duplicate samples held at -20°. Lowering the temperature 23° brought about a decrease of 12.5 in percentage of water unfrozen. The decrease in unfrozen water from -5° to -20° on this date amounts to approximately 22.5 per cent as calculated from fig. 4, page 38. Adding these two values gives a total decrease of 35.0 in percentage of water unfrozen in a temperature drop from -5° to -43°C. In other words, the unfrozen or "bound" water decreased very decidedly with temperature lowering.

Jones and Gortner (24) found the quantity of unfrozen water in gelatin gel to change very little, if at all, between -6° and -50°C., while in silica gel and other gels of the inelastic type unfrozen water decreased with temperature lowering. Again there is evidence that the behavior of the substances responsible for retaining water unfrozen in apple shoots is similar to the inelastic type of gel rather than the elastic type.

It is possible that during January a temperature of -20° is not cold enough to test critically the capacity of a shoot to survive cold. Hildreth (18) showed that a tender apple variety was killed by a temperature of -41°C. in January, while a hardy variety survived this temperature.

Since the data in table 8 show an increase in ice formation with temperature lowering, it is suggested that perhaps a more satisfactory temperature at which to measure the ability of a shoot to retain water unfrozen would be one nearer the killing point. At this critical point the quantity of water unfrozen would probably be at a minimum for survival. Varieties falling below their minimum requirement would die, while varieties retaining their hydration point above their minimum level would survive.

Assuming that the minimum water requirement for survival is the same for the protoplasm in all varieties, separation of tender and hardy varieties would be possible then by measuring their capacity to retain moisture unfrozen at this point.

It can be seen in table 8 that at -43° the percentages of unfrozen water do not allow the varieties to be placed in order of hardiness on this basis. Not only is this impossible using percentage of water unfrozen as a basis, but also when the unfrozen water is expressed as weight in grams, as a percentage of dry weight, or as a percentage of the fresh weight of the sample.

It may be that the assumption of minimum water requirement for survival is incorrect, or perhaps the varieties differ in this requirement. On the other hand, the water relations in the protoplasm or the living portions may be masked to a large extent by other materials present in the tissue. Even though a separation of hardy from tender varieties was not possible at this low temperature, it still appears to be the logical point at which to compare capacities to retain water unfrozen. The fact that death occurs at the lower temperatures where the quantities of water remaining unfrozen become smaller and smaller indicates that a relationship exists between death and the quantity of water retained against freezing.

Effect of a preceding warm spell on unfrozen water

It is generally accepted that injury from cold will result if a warm spell during the winter is followed by low temperatures which would not have been injurious in the absence of the mild period. Injury of this nature is especially prevalent on peaches and plums in a region where the temperature fluctuates over a wide range during the winter. Under such conditions some alteration in the water relationship should occur if the injury is the result of water loss in the formation of ice.

To measure the influence of a warm period as it affects the quantities of water unfrozen, four shoots of each variety were collected on Jan. 13, 1933. In two of these unfrozen water was measured immediately so that the variation between individual shoots could be noted and also to have a basis for comparison with the treated samples. One set of the remaining shoots was placed with the cut ends in water in a cold storage room at $0^{o}C$. The other set remained in the laboratory at room temperature until Jan. 24, when unfrozen water was measured in both at the same temperature as the first two sets, $-20^{o}C$. The data collected from these observations are shown in table 9.

It is obvious from the data in table 9 that the two samples measured on Jan. 13 were very much alike in their water relations. In both of the stored samples there was a noticeable increase in the percentage of water, as is evident from a comparison of their means with the two samples not stored. Notwithstanding this increase in total water, the quantity of water remaining unfrozen in the samples stored at

Table 9. The effect of a previous warm spell on water retaining capacity against freezing.

Variety	Jan. 13, 1933				Jan. 24, 1933			
	Sample I		Sample II		Room		0°	
	Percentage of				Percentage of			
	water	unf.w.	water	unf.w.	water	unf.w.	water	unf.w.
Hibernal	47.5	43.6	46.3	41.5	53.3	37.9	51.4	37.7
Virginia	51.6	36.0	51.0	37.5	57.0	27.1	53.9	34.8
Dudley	50.2	39.4	45.8	45.5	56.1	29.7	51.5	38.9
Wealthy	47.4	41.7	47.4	45.2	54.8	29.3	50.8	40.2
Ioensis	48.8	41.2	47.4	41.9	53.6	32.1	52.5	38.7
Delicious	45.7	42.6	50.2	35.5	56.4	30.5	52.6	36.3
Stayman	46.7	39.8	47.1	42.0	54.0	30.4	52.2	36.4
Mean	48.3	40.6	47.9	41.3	55.0	31.0	52.1	37.6

Comparisons	Values of "t"
Sample I and Sample II	0.50
Room and Sample II	4.75
0° and Sample II	0.53
Significant	2.14
Highly Significant	2.98

0° is not significantly different from that in set II. This lack of significance between the two sets is indicated by the small value of "t" (0.5) and suggests that no change in the water retaining capacity against freezing occurred during the storage period at 0°. (The test for significance in this experiment was made on grams of unfrozen water and not percentages.)

On the other hand, a comparison of the unfrozen water in the set stored at room temperature with that in sample II shows a marked alteration in capacity by the storage treatment. The significance of the difference between these two sets is indicated by the large value of "t", 4.75.

It may be concluded, therefore, that the storage at room temperature decreased the water retaining capacity of the shoots, while the samples held at 0^{o} were not changed appreciably in this capacity. It is true that the conditions under which the shoots were stored in this test are seldom duplicated in nature; nevertheless very similar conditions are often observable and it seems probable that the results are indicative of what occurs during a warm period in winter. If this assumption is correct, the data might be used to further the hypothesis of the association between water retaining capacity and ability to survive cold.

Effect of heat treatment on unfrozen water

It was stated previously that the influence of other component materials in the shoots might mask the water relationships of the protoplasmic portion of the tissue. An isolation of the water retaining capacity of the protoplasm alone would be desirable because the vital activities are assumed to be limited to this portion. By ascertaining the total quantity of water unfrozen in a living shoot and the quantity held after killing the shoot, it might be logical to attribute the difference in these two values to the influence of the living protoplasm. An attempt to measure this influence was made with the following procedure.

One set of a pair of duplicate samples was heated in a water bath at 80°C. for one hour. At the end of the hour this set with its duplicate was held in the bath at -20° for 4 hours, after which the unfrozen water determination was made. By this treatment it might be expected that the protoplasm would be disorganized by the heat and consequently lose its ability to retain water against freezing. Curves for the mean values of 15 varieties are plotted in fig. 2, page 38. For convenience in comparison the curve for the unheated duplicate samples is plotted in the same graph.

It is interesting to note (Fig. 2) that instead of the anticipated decrease in unfrozen water by the heat treatment there was actually an increase during most of the year. Throughout the spring and summer months the curve for heat-treatment is above that of the untreated samples, but upon the approach of winter it decreases until in December when it shows a slightly lower value for unfrozen water than is exhibited in the untreated duplicates. This lower value continues through the winter until the month of March, when the heated samples again retained more water unfrozen.

The relative position of the two curves is probably correctly explained by a sugar-starch relationship. Much work has

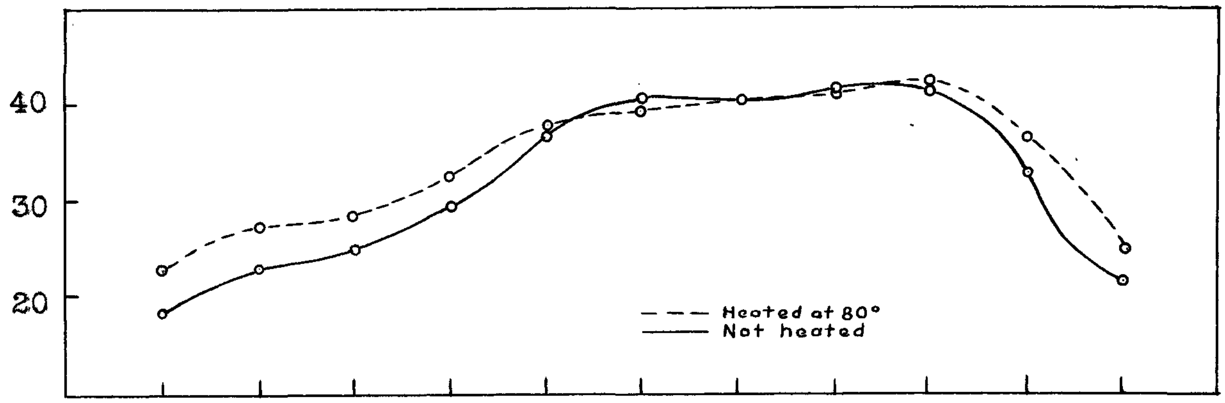


Fig. 2. Effect of heat treatment on unfrozen water, 1931-32.

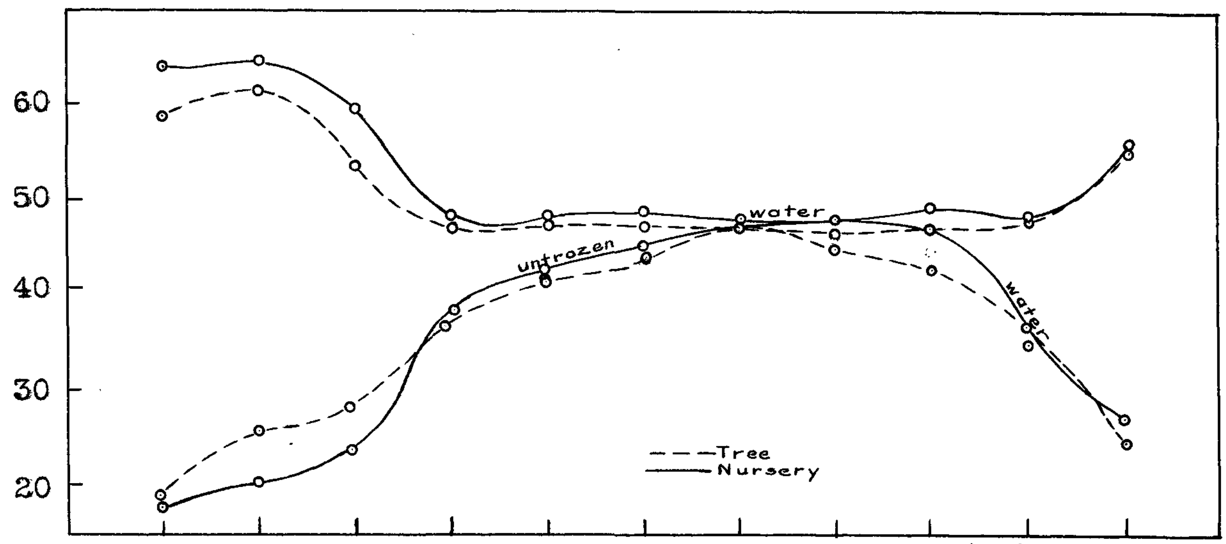


Fig. 3. Percentage of total and unfrozen water in tree and nursery shoots, 1932-33.

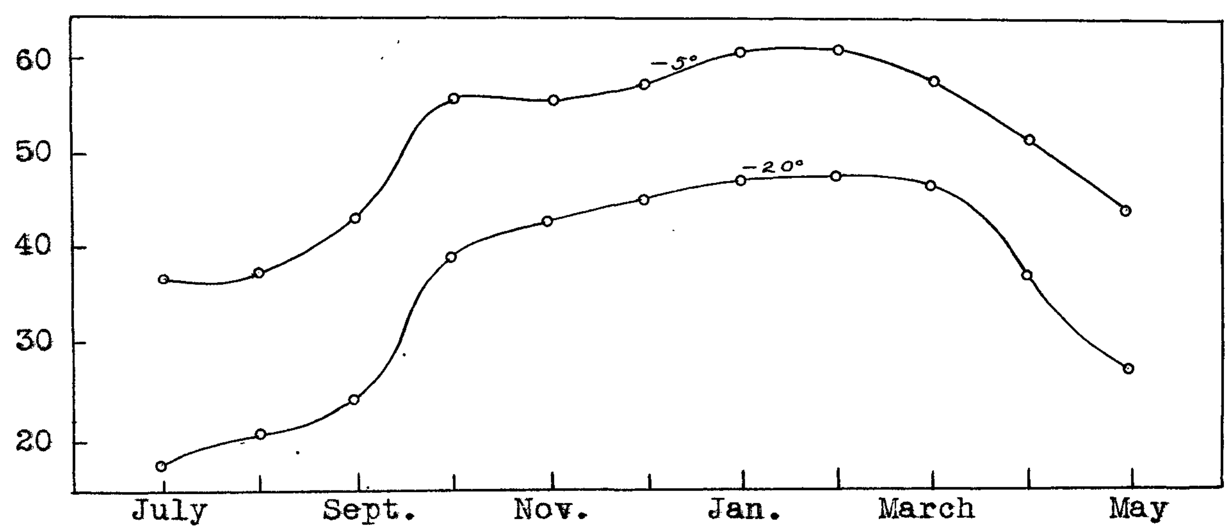


Fig. 4. Percentage of unfrozen water at -5° and -20°, 1932-33.

been published (9), (15), (18) to show that apple shoots increase in sugar content and decrease in quantity of starch during winter. It is also well known that heat augments the imbibitional properties of starch. Thus, during the season when starch is present in largest quantities the percentage of unfrozen water is greater in the heat treatment. When the starch supply falls off in early winter this influence through heat is no longer observable. At the return of spring, however, the presence of starch is again marked by an increase in percentage of unfrozen water in the heated samples.

Why the curve for heat-treatment should fall below that of the untreated sample in winter is not clear, but it is suggested that the heat-treatment had some additional influence on the materials other than that attributed to starch.

It is hardly necessary to point out that it was impossible to measure the influence of the protoplasm in the retention of water against freezing with this procedure.

Effect of oven drying on unfrozen water

There was discussed in the previous section an unsuccessful attempt to ascertain the importance of the living protoplasm in retaining water against freezing. The measurements made there were on fresh material that had been heated for one hour. In this section the results from measurements made on the same samples before and after oven drying are discussed. Before making the second measurement the dried samples were soaked 22 hours in distilled water.

It was thought that a thorough oven drying would certainly disrupt the colloidal organization in the tissue beyond recovery and thus the difference in unfrozen water between the original sample and that in the dried sample should give some idea of the importance of the inert materials in the retention of water against freezing.

Some results of the determinations are shown in table 10.

Table 10. Effect of oven drying on water relationships.

Variety	Fresh		Soaked	
	Percentage of			
	water	unf.w.	water	unf.w.
Hibernal	49.9	36.5	38.6	26.3
Virginia	50.1	35.6	39.7	27.1
Dudley	49.3	35.5	38.4	24.9
Wealthy	49.4	34.9	37.6	24.6
Ioensis	47.8	36.6	35.9	26.5
Delicious	51.2	33.7	41.1	27.2
Stayman	46.8	32.2	41.3	24.9
Mean	49.2	35.0	38.9	25.9

It can be seen in table 10 that after the samples were dried they did not reabsorb in 22 hours as much water as was present in the original fresh tissue. A larger mean of 10.3

in percentage of total water in the fresh samples suggests that an alteration in the water relations of the substances composing the tissue was brought about by drying. Not only is this effect noticeable in the total water reabsorbed but also in the capacity to retain this water against freezing. As expressed on a dry weight basis the fresh samples held 9.1 per cent more water against freezing than was retained in the same set after oven drying. It would probably be assuming too much to attribute all of this larger water retaining capacity to the colloidal organization of the living tissue, yet it is possible that this organization plays a major role in this respect.

The importance of the inert material in preventing the water from freezing is disclosed by a mean of 25.9 for unfrozen water in the soaked samples. Evidently the largest fraction of water remaining unfrozen at -20° serves merely for hydration of the lifeless materials.

Comparison of unfrozen water in nursery and tree shoots

Some question might arise as to the behavior of the water relationships in the same variety when grown under different conditions. It is quite well known that the hardiness of a tree can be influenced by cultural practices. Perhaps the water retaining capacity of a variety varies with the conditions under which it is grown.

To test this influence of environment upon the water retaining capacity in shoots of the same variety from different sources, unfrozen water measurements were made at the same time on samples from nursery and from orchard trees.

Although it is unnecessary to include data for individual varieties, the means of the same 10 varieties from each source are plotted in fig. 3, page 38. An inspection of the curves reveals a marked difference in the water relationships of the shoots from the two sources. In the tree shoots there is a more gradual maturing of the wood in the fall as indicated by its lower percentage of water unfrozen. This slower change in quantities of unfrozen water is noticeable again in late winter and spring. Not only are the rates of change less abrupt in the shoots from the trees but also of less intensity, reaching the higher level of the nursery shoots only once during the winter. This lower level for unfrozen water in the tree shoots is surprising in view of the lower percentage of total water present and is probably indicative of a less complete maturity than that characterizing the shoots from the nursery.

These differences in the same varieties grown under unlike conditions suggest that physiological as well as inherent characteristics are important in determining water relationships in the shoots.

Unfrozen Water as a Means of Separating Hardy from Tender Varieties

Comparison of varieties frozen at -20°C.

One of the principal objectives of this investigation was to ascertain the usefulness of unfrozen water measurements for separating apple varieties on the basis of their winter hardiness. Assuming that the capacity of a variety to retain moisture in the unfrozen state when subjected to freezing temperatures is related to its ability to survive cold, then it is logical to suppose that the hardier varieties would have the larger capacity in this respect. In this connection, however, the rate and time of maturity of the different varieties are factors that might influence the state of hardiness as well as the quantity of water held unfrozen. Granting such a possibility, it would seem desirable to have a knowledge of the changing water relations from season to season throughout the course of the year. Accordingly, unfrozen water measurements were made at monthly intervals on shoot growth of the current season. By this procedure any distinguishing varietal characteristics such as rate and time of maturity, as indicated by their water relationships, might be disclosed along with the relative water retaining capacities of the different varieties.

Although curves are shown for only three varieties, the

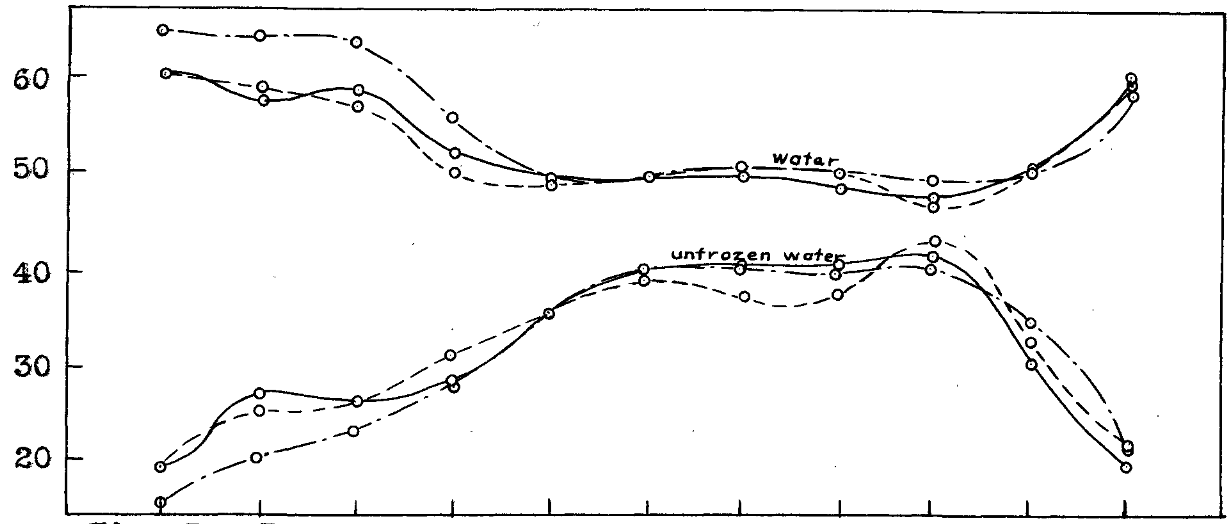


Fig. 5. Percentage of total and unfrozen water, 1931-32.

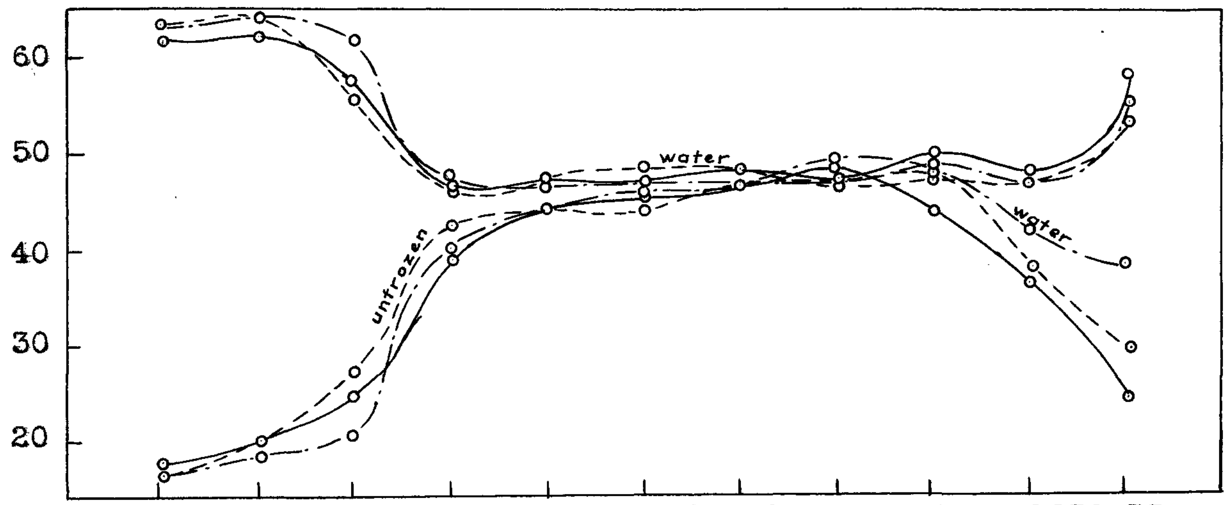


Fig. 6. Percentage of total and unfrozen water, 1932-33.

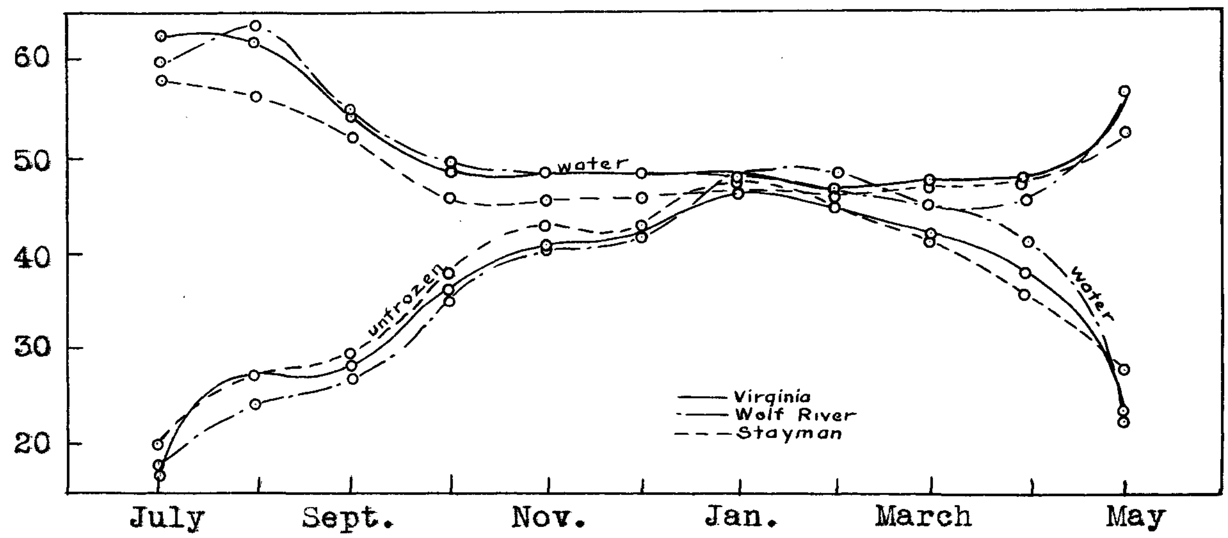


Fig. 7. Percentage of total and unfrozen water in tree shoots, 1932-33.

data for all fifteen have been plotted and carefully studied for any differences that might allow for their separation into an order of hardiness. The curves in figs. 5, 6 and 7 are plotted with data from the varieties Virginia, Wolf River and Stayman; representing the two extremes and a mean in the scale of hardiness as observed from years of horticultural field experience (27). Virginia is exceptional in its hardiness, Stayman is decidedly lacking in this respect, while Wolf River is intermediate in ability to survive cold.

An examination of the graphs in figs. 5, 6 and 7 will show that the curves of the three varieties are very similar in percentages of unfrozen water. Apparently there is no relation between the known hardiness of a variety and its ability to retain water unfrozen at -20°C. When the data for all of the 15 varieties are plotted together, the interlacing of the individual curves makes it quite impossible for any variety to be distinguished by the position of its curve at any time. In other words, the order of hardiness as arranged on the basis of unfrozen water varies from time to time with no variety maintaining a consistently higher or lower position in the scale. This is true whether the unfrozen water is expressed as a percentage of total water, of dry matter, or on a fresh weight basis. That is, the unfrozen water in apple shoots held at -20°C. for 4 hours will not serve as a basis for separating a hardy from a tender variety, regardless of the time of the year at which the measurement is made.

Perhaps this conclusion should not be surprising, in view of the fact that Dunn (8) and Hildreth (18) found the survival order among varieties to change from time to time when apple shoots were frozen and allowed to recover. Similar fluctuations have been noted in other plants. Even so, Nichols and Lantz (38) and Dorsey (6) have observed in apple trees that the degree of browning after winter injury is quite consistent for a variety. It should be pointed out, however, that the degree of browning is not a measure of survival since many shoots may be severely browned and yet recover apparently uninjured.

Statistical analysis of the data

Even though it is impossible to separate varieties into an order of hardiness, a statistical analysis of variance indicates that varieties differ significantly in their capacities to retain water against freezing. Results of the analysis are shown for the tree shoots as well as for the nursery shoots for both years in table 11.

In the test for significance of varietal differences it is necessary to compare the value (1429) listed under "mean square" with that of "interaction" (203) in the same column. In like manner the significance of the difference between months may be tested. By these tests there was found in all

Table 11. Analysis for variance in varieties tested.

	Nursery shoots				Tree shoots	
	1931-32		1932-33		1932-33	
	D.F.	Mean square	D.F.	Mean square	D.F.	Mean square
Total	329	1412	359	2612	219	2375
Within pairs	165	70	180	107	110	139
Between means of varieties	14	1429	14	920	9	1811
Between means months	10	40463	11	76663	10	44977
Interaction or experimental error	140	203	154	404	90	431

three series a highly significant difference between varieties. Tests for significance were made in accordance with Fisher's method, but using the tables for "F" given by Snedecor (50). This is statistical evidence that varieties do differ significantly with respect to their water retaining capacity against freezing even though it is not possible to arrange them into an order of hardiness on this basis. In spite of the fact that this analysis does not explain at what time or where the differences occur, it does prevent the mistake of concluding that varieties are all alike in this respect. The difference in unfrozen water between months is more obvious than that between varieties and is also proven highly significant by the same test. This significance would be expected from an inspection of the curves.

A point discussed previously in regard to errors of technique and sampling is shown clearly in table 11. Values listed after "within pairs" are indicative of the error resulting from laboratory technique alone, while those under "interaction" represent the biological error plus the error of laboratory technique. This latter combination is the best estimate of the "experimental error." Snedecor (50) has discussed in some detail the calculation and the significance of these two errors.

Comparison of varieties frozen at -5° and -20°

It has already been shown that the formation of ice in apple shoots increased with temperature lowering. Such a relationship indicates that part of the water freezes very readily, while other portions form as ice only at lower temperatures. A further cooling after the first part is frozen would freeze that water which is retained more tenaceously and is removed only by the lower temperature. A test of the capacity of a shoot to retain water between a high and a low freezing temperature should give some measure of its ability to survive cold; since it is this last portion, held more firmly, that decides survival or death through freezing.

In determining the quantity of water removed between a mild and a more severe temperature, unfrozen water measurements were made in duplicate samples held at -5° and -20°. The curves for the means of the 15 varieties are presented in fig. 4, page 38. These curves are shown merely to give some idea of the general course of unfrozen water throughout the year.

It is noted that the spread between the two curves is greater in the summer and autumn than during the winter months. In May of the next spring this tendency is again noticeable. The significance of this larger spread during the seasons of least resistance to cold is not exactly clear, but it suggests that less alteration of the water relationship is effected by a lower temperature during the period of greatest resistance.

In comparing the individual varieties the differences in percentage of water unfrozen between the two temperatures were averaged for December, January, February and March, the months of greatest resistance to cold. These averages are listed in table 12.

It is obvious from table 12 that the varieties cannot be arranged in the proposed order of hardiness from the means of the differences in percentage of water unfrozen. If a line is drawn under the value for Wolf River, however, it may be seen that varieties falling below the line have larger mean differences than those above. With the exception of Hibernal and Wagner the varieties fall definitely into two distinct groups. Comparing the two groups statistically gave a value for "t" of 5.41, with 3.01 as the highly significant value. This is statistical evidence that a tender variety can be distinguished

Table 12. Mean differences in unfrozen water between -5° and -20°.

Variety	Order of hardiness	Mean difference in percentage of water unfrozen	Order of differences
Hibernal	1	12.0	8
Virginia	2	10.7	3
Shield's	3	9.9	1
Dudley	4	11.1	5
Okabena	5	10.9	4
Wealthy	6	11.6	6
Ioensis	7	9.9	2
Wolf River	8	11.8	7
Cortland	9	13.4	11
Baltimore	10	13.8	12
Jonathan	11	14.9	14
Delicious	12	13.0	10
Grimes	13	13.9	13
Wagner	14	12.1	9
Stayman	15	14.9	15

from a hardy one by the larger quantity of ice formed in its shoots during a temperature drop from a mild to a more severe freezing.

It may be somewhat disappointing to find that the varieties cannot be placed in a more definite order of hardiness, using the unfrozen water values as a basis for arrangement, but it should not be forgotten that this order may vary from time to time. Even if an exact arrangement is not possible by following this procedure, it is of some value to be able to place a variety in a tender or hardy class irrespective of its relative position. Although the evidence is in favor of such a separation, the two exceptions and the arbitrary position of the dividing line must not be overlooked in forming conclusions.

Other comparisons of varieties

In addition to the comparisons just discussed some other procedures were employed in an effort to separate the hardy from the tender varieties.

Robinson (42) found that it was the increase in capacity to retain water against freezing, after a hardening treatment, that distinguished a hardy from a tender species of insect. His findings would naturally suggest a similar comparison in this material.

In order to measure this increase in capacity the unfrozen water values for July were subtracted from those of January. The difference between the two values was taken as the increase in unfrozen water from the tender to the hardened condition. Results for the two years were similar in not allowing a separation of the hardy from the tender varieties on the basis of increase in percentage of water unfrozen.

Another comparison of the varieties was made from the unfrozen water of the samples that were heated one hour at 80°C. and frozen at -20°C. It is quite unnecessary to present any varietal data from this treatment, but it should be mentioned that no separation of hardy and tender varieties was possible from the results obtained.

A similar unsuccessful attempt resulted from the measurements made at -5°. These data are omitted also but may be found in the appendix. In summing up varietal comparisons it should be stated that all of the data obtained have been studied for characteristics that might serve to separate the varieties on the basis of their hardiness. With the possible exception of the observations presented in table 12, no results were secured that would allow a hardy variety to be distinguished from a more tender one.

The Course of the Water Relationships Throughout the Year

For a general discussion of the course of the water relationships throughout the year the means of the 15 varieties are plotted in figs. 8 and 11. Charts for the temperature and rainfall over the same period accompany these curves. Rainfall is illustrated as total precipitation per week, while the points on the temperature curves are determined as means of 10-day intervals for maximum and minimum temperatures as recorded by the United States Weather Bureau at Ames, Iowa.

At the beginning it should be emphasized that each point in figs. 8 and 11 is the mean of 15 varieties. With this number of observations a small difference in location of the point is likely to be of more significance than if the position were established by a single pair of observations of one variety.

These curves represent data from shoots of a single growing season. Measurements were made at monthly intervals throughout the year. All new growth present in the spring months was removed before the samples were cut. In this way the changes observed are not attributable to the presence of new succulent tissue but rather to alteration of the material of the same source as that studied through the preceding summer and winter.

On most varieties buds were just beginning to enlarge on April 21 in 1933, and leaves were only partly developed on April 21 in 1932. As observed from material in the field there is apparently no relationship between the hardiness of a variety and the time its buds break in the spring. Nevertheless the stage of development in the buds is reflected by the water relations in the shoots. The data obtained on April 21, 1933 showed that varieties having buds ready to break retained a smaller percentage of water unfrozen than those varieties exhibiting a less advanced stage of bud development.

This alteration in water retaining capacity with spring growth is probably best shown by the abrupt drop in unfrozen water from March to April, fig. 11. This sudden change in water retaining capacity against freezing takes place in spite of a slight decrease in the total quantity of water present. A similar decrease in percentage of water unfrozen is also noticeable in the spring of the previous year (Fig.8).

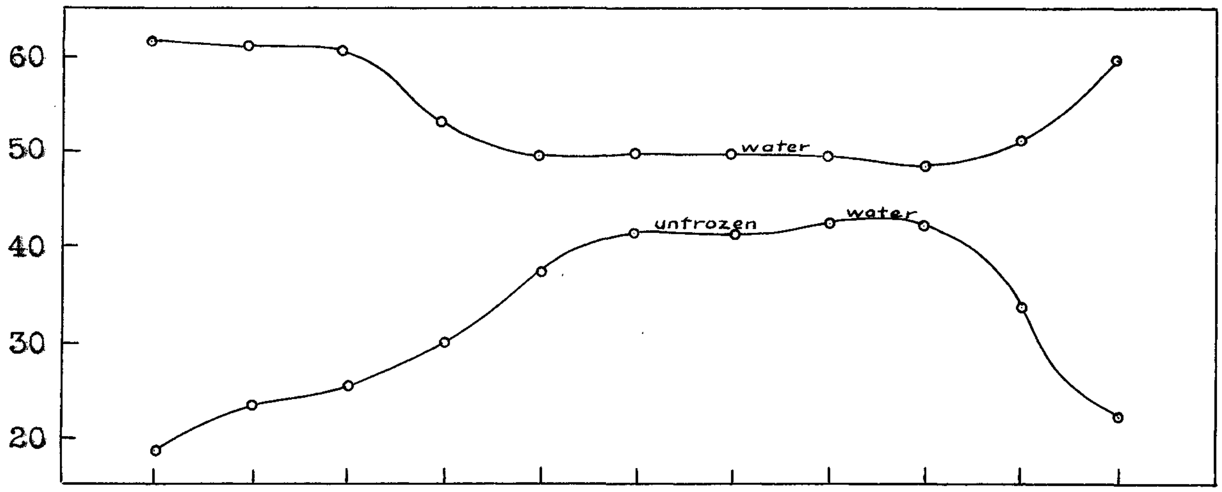


Fig. 8. Percentage of total and unfrozen water in nursery shoots, 1931-32.

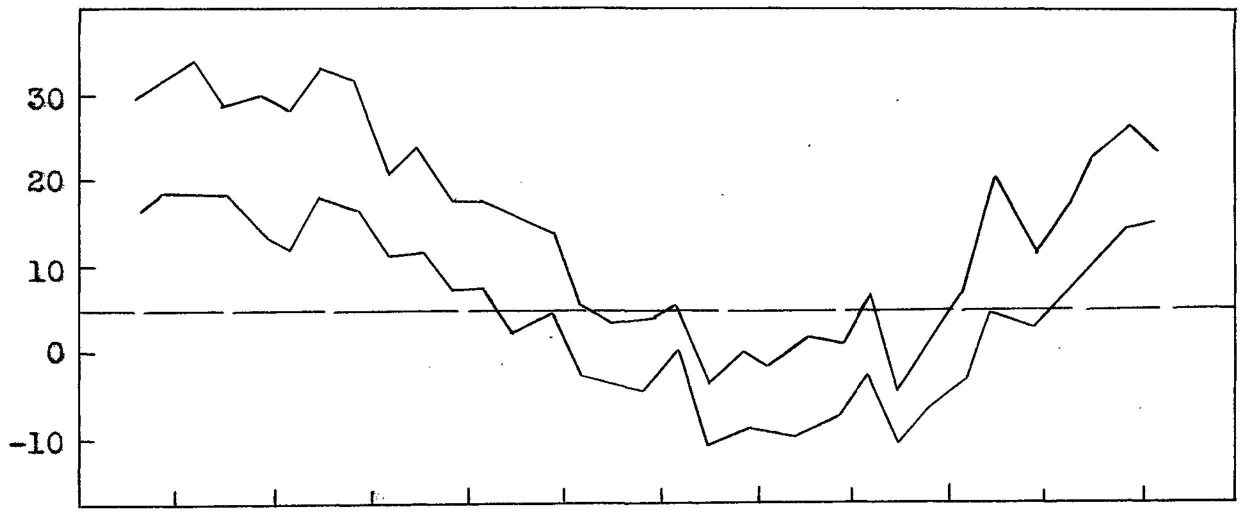


Fig. 9. Maximum and minimum temperatures, 1931-32.

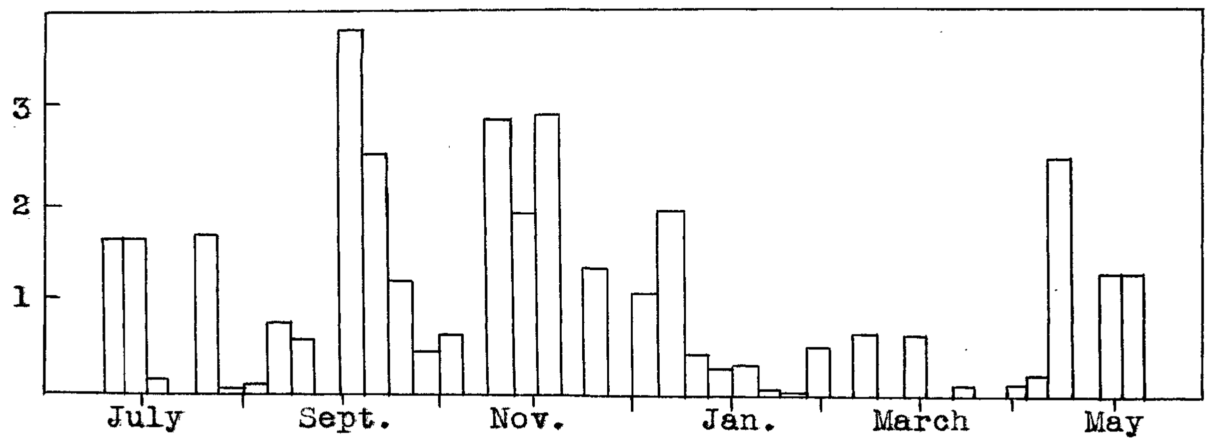


Fig. 10. Precipitation, 1931-32.

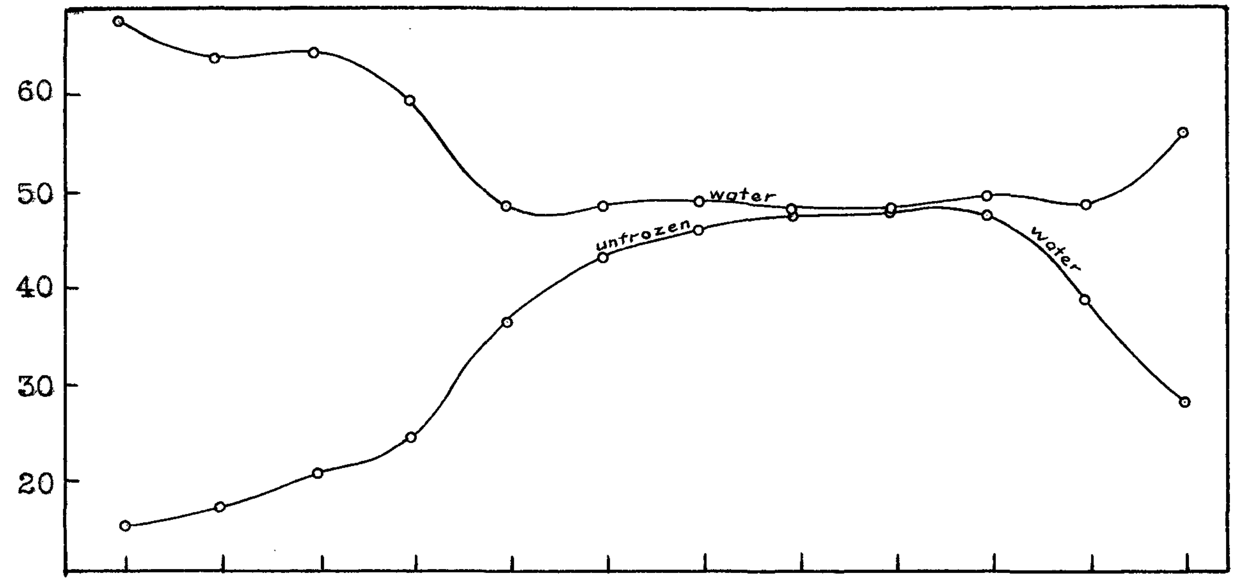


Fig. 11. Percentage of total and unfrozen water in nursery shoots, 1932-33.

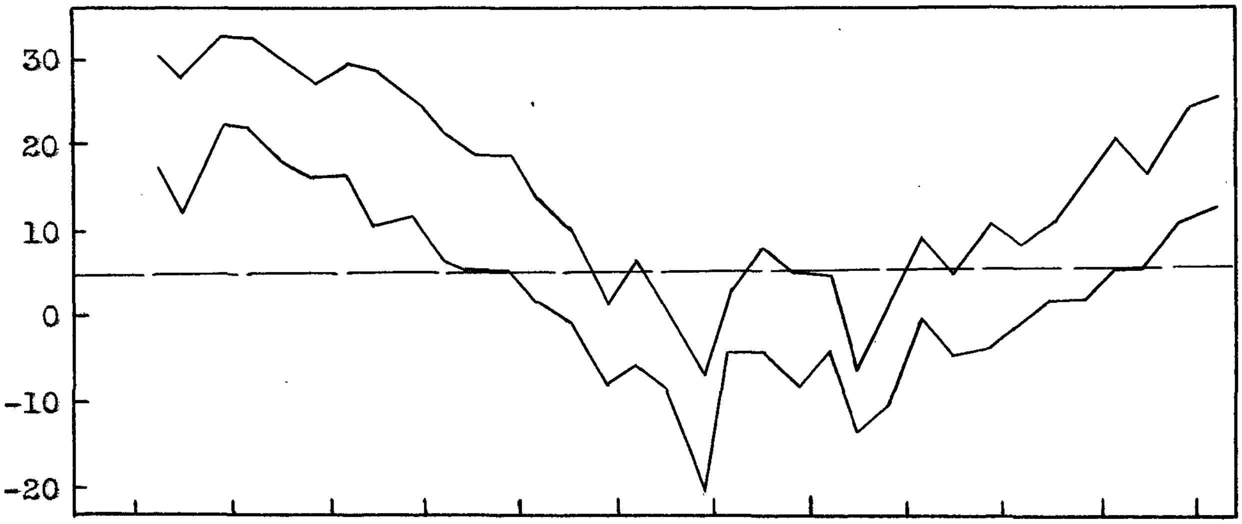


Fig. 12. Maximum and minimum temperatures, 1932-33.

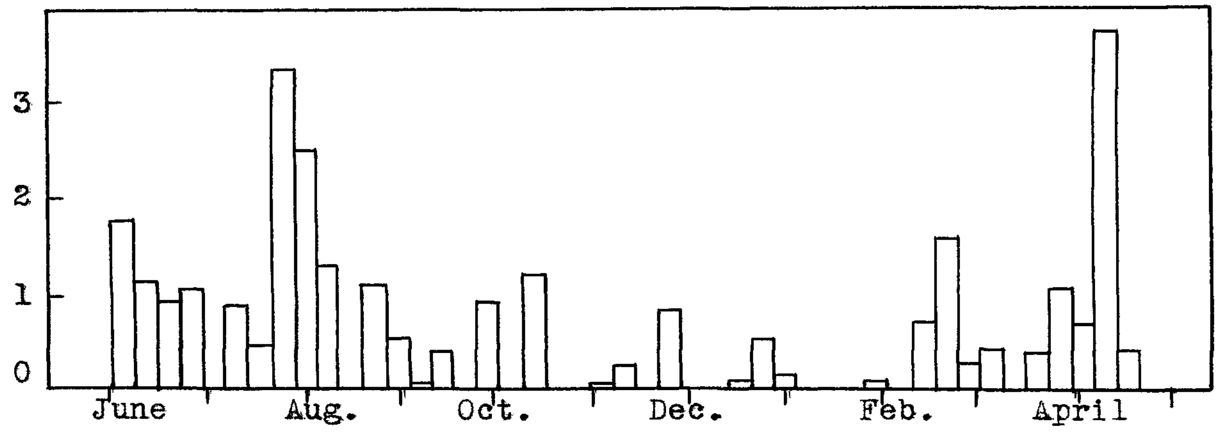


Fig. 13. Precipitation, 1932-33.

Another change in the water retaining capacity of the tissue is noticeable in the autumn and early winter of each year. In early winter this change also takes place quite independently of the total water content of the shoots. Undoubtedly the cessation of food synthesis took place long before November, when many of the leaves had fallen and the remainder were dead from frost. Still in both years there is a gradual increase in capacity to retain moisture unfrozen until March. This increase proceeded even after the water content in the tissue had reached the constant level maintained throughout the winter.

With the exception of the two examples just discussed the quantity of water remaining unfrozen appears to be rather closely related to the total water content of the tissue. This is shown by the fact that a low percentage of unfrozen water usually accompanies a high moisture content, while a high water retaining capacity characterizes shoots having a low percentage of water. If the unfrozen water was merely a function of the total quantity present, it would remain unchanged after the water content of the shoots reached a constant level. In general, this is the case, but the two exceptions noted in the spring and autumn furnish evidence that there must be some other influence on water retaining capacity besides the percentage hydration of the tissue. In the autumn the exception is that of an increase while in the spring that of a decrease in capacity. These exceptions indicate that at least part of the substances retaining water unfrozen are associated with the vital activities of the tissue. If such were not the case it would be difficult to account for these alterations in capacity without an accompanying change in quantity of matter present. It is concluded, therefore, that living processes have an influence upon the substances responsible for retaining water unfrozen. This conclusion is contrary to that of Meyer (31) cited previously.

Of course it is impossible from the data on hand to account for the reactions responsible for this alteration in water retaining capacity. It is suggested, however, that the protective action of sugar may be of some importance in this respect. Especially in the light of a recent publication by Newton and Brown (36) does the influence of sugar seem possible. They found in freezing expressed plant juices that less precipitation of proteins occurred after sugar had been added than when it was omitted. If such protective action existed in apple shoots, one might expect a maximum in water retaining capacity during the time when sugar is known to be present in largest quantities. In an analysis of apple shoots Hildreth (18) discovered that the highest sugar content occurred during the winter months. At this same time the water retaining capacity against freezing is at a maximum, as seen in figs. 8 and 11. The occurrence of the two maximums at the same time may be a mere coincidence, but there is a possibility of an interrelationship.

Along with the protective action of sugar it is possible that alterations in the materials present may cause some change in water retaining capacity. In autumn synthesis of substances into more complex structures could alter the physical and chemical state of the matter present without appreciably changing the dry weight. In the spring these same substances could be changed in the reverse direction by an analysis or breaking down process. This change in state of the matter on hand could account for the alteration in water retaining capacity without a measurable increase or decrease in percentage of dry matter.

Some unpublished observations of Martin (28) are of interest here. In a study of hardiness of sweet clover over a number of years, he has found repeatedly through cytological technique that the protoplasm in the cells of the roots appeared very dense at the approach of winter. With the inception of growth in the spring he observed an abrupt liquification of the protoplasm. The exact time at which these changes took place varied with the weather conditions in late autumn and early spring. Loomis (25) has suggested that hardiness is the result of a structural differentiation of the protoplast which makes it more resistant to precipitation, such differentiation being dependent upon and in part initiated by a high sugar concentration in the tissue. These observations appear to be in agreement with the results of this study, but it should be mentioned in opposition to this view that Harvey (16) believes the process of hardening is accompanied by an analysis rather than a synthesis of substances. He found more decomposition products of proteins in cabbage after hardening than were present in the unhardened leaves. Newton and Brown (36), however, were inclined to attribute the presence of such intermediate products to the action of freezing and not to the hardening treatment.

No matter what the explanation underlying the changes in water retaining capacity may be, it seems clear that it is associated with or at least parallels the hardiness of the shoots. Hildreth (18) has plotted killing temperatures for apple shoots over the course of a year. His data show a killing temperature of -3°C. in July, with a gradual lowering to about -40° at the first of January. Little change is then noted until an abrupt upward trend begins the first part of April, reaching -6° in May. The striking relation between Hildreth's killing temperature curve and the curves for unfrozen water in this study suggests that the ability to retain water against freezing may be of importance in cold survival of apple shoots.

The greater resistance during the winter months is associated with a larger water retaining capacity at this time. The same freezing temperature is able to remove as ice a much smaller percentage of water during the period of greatest resistance than when the tissue is more readily killed by cold. This smaller portion removed as ice would mean less departure from the normal water relationships in the tissue. It may be that it is necessary to remove as ice a rather definite percentage of the total water present before serious injury or death of the protoplasm results. Under such a condition the shoots in the winter could withstand a much lower temperature before this minimum percentage would be reached.

Too much emphasis should not be placed on the percentage of water unfrozen without a discussion of the percentage of total water. Numerous investigators (1), (7), (23), (35) and (55) have pointed out that hardiness is associated with a low moisture content. Some authors (22), (48) have contended that hardy varieties can be separated from tender sorts by their lower moisture content. Such is not the case with the apple shoots used here, although there is evidence that the degree of resistance to cold is related to the water content of the tissue. This relationship is readily seen from an inspection of the percentage water curves in figs. 8 and 11. From summer to winter there is a marked decrease in the moisture content of the shoots. During the winter the percentage of water reaches a low level that remains quite constant until spring, when it rises very abruptly. It is obvious that when the shoots were least resistant the moisture content was high, while during the period of greatest resistance to cold the percentage was low.

A comparison of the two years shows that the percentage of water in the shoots was higher during the winter of 1931-32 than in the following year. A lower percentage of unfrozen water is also observable in 1931-32. The fact that the two years differ with respect to the position of their curves is probably correctly explained by the comparatively warm, moist autumn of 1931, in which some woody plants bloomed in late fall and many exhibited a renewal of growth at this time. A glance at the temperature and rainfall charts (Figs. 9, 10, 12 and 13) will show the difference between the two autumn seasons.

From past experience with winter injury to fruit trees it is known that the conditions of 1931 were not conducive to a thorough hardening as contrasted to the weather of 1932. Dexter (5) and Tumanov (57) have found recently that conditions favoring food accumulation are generally favorable for the hardening of plants. The season of 1931 was not especially favorable for the accumulation of foods as compared with the following autumn. It might be assumed, then, that the shoots were less hardy during this year than during the following year; an assumption that is supported by the smaller percentage of unfrozen water as well as total moisture content in the shoots.

Another relationship between the course of the curves for unfrozen water and climatic conditions is suggested by an investigation by Harvey (17). In a study of elm seedlings he determined the threshold value for hardening to be about 5°C.

when the exposure was continuous. Little or no hardening was observable at 10°C. In figs. 9 and 12 it may be noted that the 5° line intersects the maximum temperature curves in the spring of both years at about the same time the drop in unfrozen water occurs. The influence of a warm temperature on the ability to retain water unfrozen was pointed out in a previous section and those results are apparently substantiated by a similar decrease observable here.

DISCUSSION

In attempting to separate apple varieties into an order of hardiness it should be remembered that many factors must be considered. In the first place, the order of hardiness probably varies from time to time with no variety maintaining the same relative position throughout the year. Not only is there a variation between varieties but also the shoots of the same variety respond differently to the same freezing temperature. Different degrees of injury through cold may occur without death resulting to the entire shoot. It is only when a sufficiently large portion of the living tissue is killed that recovery becomes impossible. The magnitude of this portion may vary among varieties and from shoot to shoot within a variety.

Again there is a difference in the same variety brought about by the external environment. The hardiness of a tree will vary with climatic and other conditions quite independently of its inherent resistance to cold.

In spite of these sources of variation hardy varieties survive winters that kill the more tender sorts. It is this constancy of behavior that suggests some inherent difference in the hardiness of the varieties.

To test this inherent difference by measuring the capacity to retain water unfrozen is complicated by the factors mentioned above, plus the lack of information as to the necessity of this capacity in survival. The rate of freezing and the rate of thawing have both been shown to have an influence on the injury resulting from low temperatures. Neither of these affect measurably the quantity of water frozen. There are probably other factors influencing the amount of injury without altering appreciably the quantity of water frozen in the time. It is these additional influences that demand caution against placing too much emphasis upon capacity to retain water against freezing as the major factor in surviving cold. Nevertheless the importance of this capacity is evidenced again and again in the study presented here. This is especially noticeable in the comparison of unfrozen water in the tips and bases of the same shoots, in the effect of period of exposure to freezing temperatures, in the effect of extreme cold, in the effect of a preceding warm period, in the comparisons at -5° and $-20^{\circ}C$. and in the general course of unfrozen water throughout the year.

The attempts to separate the hardy from the tender varieties using unfrozen water as a basis were futile, with perhaps the one exception of the comparison made at -5° and -20°. In this case the tender varieties, taken as a group, exhibited a greater loss of water in formation of ice between the two temperatures than did hardy varieties. Although the data indicate that such a procedure might serve for placing a variety into a hardy or tender class, more evidence is necessary to substantiate this point before a positive assertion can be made.

SUMMARY

Unfrozen water measurements were made on apple shoots by the heat-of-fusion method at monthly intervals throughout the year.

Some evidence was obtained to show that the freezing process in apple shoots was partially reversible, resembling the behavior of an inelastic gel in this respect.

In general the data supported the hypothesis that the capacity to retain water against freezing is associated with winter hardiness of apple shoots.

A statistical analysis of the data indicated that varieties differ in their capacity to retain water against a freezing temperature of -20°C. Nevertheless it was impossible on this basis to separate the varieties into a hardy and a tender class.

There was evidence that a tender variety might be distinguished from a more hardy one during the winter by the larger quantity of ice formed in its shoots in the temperature interval between -5° and -20°C.

A connection was pointed out between external environment and water relationships in the shoots.

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APPENDIX

Water Relations in Varieties When Frozen at - 20°C.

	July 21, 1931				August 22, 1931			
	I	II	III	IV	I	II	III	IV
Hibernal	65.3	15.9	30.0	10.4	62.8	23.9	40.6	15.0
Virginia	60.5	19.3	29.6	11.7	57.8	27.3	37.4	15.7
Shield's	59.7	19.6	29.1	11.7	61.6	22.0	35.5	13.6
Dudley	60.4	19.3	29.4	11.6	58.6	25.2	35.7	14.7
Okabena	62.8	17.4	30.5	11.0	61.6	22.5	36.2	13.9
Wealthy	58.0	20.6	28.7	12.0	60.5	24.3	37.3	14.7
Ioensis	63.0	18.7	31.6	11.7	59.5	25.0	36.6	14.8
Wolf River	65.2	16.5	31.1	10.8	64.5	20.4	37.3	13.2
Cortland	63.2	17.7	30.5	11.2	64.4	20.9	38.0	13.4
Baltimore	61.1	20.2	31.6	12.3	61.7	22.5	36.3	13.9
Jonathan	60.3	19.6	29.8	11.8	61.5	23.2	37.2	14.3
Delicious	63.5	16.6	29.0	10.6	65.0	20.3	36.6	13.0
Grimes	59.0	19.9	28.7	11.7	59.3	23.1	33.7	13.7
Wagner	63.1	17.3	29.7	10.9	58.4	27.4	38.8	16.1
Stayman	60.9	19.6	30.4	11.8	59.5	25.4	37.3	15.1

	September 21, 1931				October 21, 1931			
	I	II	III	IV	I	II	III	IV
Hibernal	57.3	30.0	40.2	17.2	51.0	34.0	35.3	17.3
Virginia	59.4	26.2	38.4	15.5	53.0	28.8	32.3	15.2
Shield's	61.4	24.1	38.5	14.8	53.8	29.2	34.0	15.7
Dudley	60.0	25.5	38.2	15.3	52.3	30.9	33.8	16.1
Okabena	62.2	23.4	38.3	14.5	53.2	29.5	33.6	15.7
Wealthy	56.0	29.3	37.4	16.4	51.5	32.5	34.6	16.8
Ioensis	59.5	27.2	40.1	16.2	50.5	34.4	35.1	17.4
Wolf River	64.1	23.2	41.5	14.8	56.0	27.9	35.4	15.5
Cortland	63.0	21.9	37.2	13.8	55.6	25.3	31.8	14.1
Baltimore	60.1	25.1	37.7	15.0	51.0	32.3	33.6	16.5
Jonathan	61.0	23.7	37.2	14.5	52.8	28.7	32.1	15.1
Delicious	62.0	23.1	37.6	14.3	55.0	27.1	33.2	15.0
Grimes	60.5	23.2	35.5	14.0	54.5	26.5	31.9	14.4
Wagner	62.2	23.7	39.0	14.7	53.1	30.4	34.4	16.1
Stayman	57.6	26.5	36.0	15.3	50.2	31.5	31.5	15.7

I-Percentage of water.
II-Percentage of water unfrozen.
III-Percentage unfrozen water, dry weight basis.
IV-Percentage unfrozen water, green weight basis.

	November 21, 1931				December 21, 1931			
	I	II	III	IV	I	II	III	IV
Hibernal	49.1	39.1	37.7	19.2	50.0	41.2	41.3	20.6
Virginia	50.4	35.7	36.4	18.0	50.1	40.4	40.6	20.2
Shield's	51.8	36.3	38.9	18.7	51.7	39.1	42.0	20.2
Dudley	49.1	40.3	38.7	19.7	48.5	44.0	41.4	21.3
Okabena	50.3	37.8	38.2	19.0	49.5	41.0	40.1	20.2
Wealthy	51.4	35.9	38.1	18.5	49.6	43.5	42.8	21.5
Ioensis	49.0	38.5	37.0	18.9	49.5	43.6	42.8	21.5
Wolf River	50.7	36.5	37.5	18.5	50.2	41.2	41.7	20.7
Cortland	49.5	36.8	36.1	18.2	50.1	41.0	41.2	20.5
Baltimore	49.9	37.2	37.1	18.6	49.4	42.0	41.0	20.8
Jonathan	49.0	36.3	34.9	17.8	50.5	40.0	41.0	20.2
Delicious	50.7	36.2	37.2	18.3	50.4	40.0	40.7	20.1
Grimes	47.2	39.1	35.0	18.5	49.6	40.8	40.2	20.2
Wagner	49.6	38.5	37.9	19.1	49.4	42.3	41.3	20.9
Stayman	49.4	35.2	34.3	17.4	50.1	39.4	39.5	19.8

	January 23, 1932				February 20, 1932			
	I	II	III	IV	I	II	III	IV
Hibernal	49.0	41.5	39.8	20.3	47.0	45.6	40.5	21.4
Virginia	50.4	41.2	41.9	20.8	49.2	41.2	40.0	20.3
Shield's	51.8	40.2	43.2	20.8	51.1	40.1	41.9	20.5
Dudley	49.0	42.7	40.9	20.9	49.2	42.3	41.1	20.9
Okabena	48.9	40.5	38.7	19.8	49.0	42.3	40.6	20.7
Wealthy	49.6	41.3	40.6	20.5	49.6	42.4	41.8	21.0
Ioensis	48.5	43.3	40.9	21.0	48.7	42.7	40.5	20.8
Wolf River	50.0	40.9	40.9	20.4	51.3	40.0	42.2	20.5
Cortland	50.3	39.7	40.0	20.0	51.0	38.8	40.4	19.8
Baltimore	47.8	43.1	39.5	20.6	49.3	42.4	41.3	20.9
Jonathan	50.5	40.1	40.9	20.2	50.1	39.5	39.6	19.7
Delicious	52.1	37.2	40.5	19.4	49.1	40.8	39.4	20.0
Grimes	47.5	41.4	37.4	19.6	46.4	45.2	39.2	21.0
Wagner	48.6	43.3	41.1	21.0	47.1	44.4	39.7	20.9
Stayman	50.9	37.6	39.0	19.1	50.4	37.8	38.5	19.0

I - Percentage of water.
II - Percentage of water unfrozen.
III- Percentage unfrozen water, dry weight basis.
IV - Percentage unfrozen water, green weight basis.

March 21, 1932 / April 21, 1932

	March 21, 1932				April 21, 1932			
	I	II	III	IV	I	II	III	IV
Hibernal	45.7	47.3	40.0	21.6	50.2	36.8	37.1	18.5
Virginia	48.7	42.8	40.6	20.8	52.0	31.9	34.5	16.5
Shield's	51.2	39.3	41.2	20.1	55.7	28.4	35.7	15.8
Dudley	49.2	41.6	40.8	20.6	51.9	33.5	36.1	17.4
Okabena	49.6	40.4	39.7	20.0	50.1	37.2	37.4	18.6
Wealthy	48.8	41.9	40.0	20.4	49.3	37.0	36.0	18.2
Ioensis	48.1	43.7	40.5	21.0	50.3	38.0	38.5	19.1
Wolf River	49.3	41.1	40.0	20.2	50.5	36.0	36.7	18.1
Cortland	47.7	42.3	38.7	20.2	-- -	-- -	-- -	-- -
Baltimore	48.4	42.0	39.4	20.3	50.9	33.3	34.5	17.0
Jonathan	48.8	40.5	38.7	19.8	53.0	30.6	34.5	16.1
Delicious	49.6	40.7	40.1	20.2	51.6	31.5	33.7	16.3
Grimes	46.9	44.7	39.5	21.0	49.7	33.0	32.7	16.4
Wagner	46.2	44.4	38.2	20.5	49.5	35.8	35.1	17.7
Stayman	47.0	43.2	38.4	20.3	51.2	33.4	35.1	17.1

May 21, 1932

	I	II	III	IV
Hibernal	61.0	21.4	33.6	13.0
Virginia	61.1	20.4	32.0	12.4
Shield's	60.6	20.6	31.6	12.5
Dudley	56.2	25.8	33.1	14.5
Okabena	60.6	20.9	32.2	12.6
Wealthy	58.6	22.4	31.8	13.1
Ioensis	57.5	24.3	33.0	14.0
Wolf River	59.0	22.8	32.8	13.4
Cortland	59.1	21.7	31.4	12.8
Baltimore	59.5	22.0	32.2	13.0
Jonathan	60.2	21.2	32.0	12.7
Delicious	60.3	21.4	32.7	13.0
Grimes	57.1	23.1	31.0	13.3
Wagner	59.7	21.7	32.3	13.0
Stayman	59.5	22.4	33.0	13.3

I - Percentage of water.
II - Percentage of water unfrozen
III- Percentage unfrozen water, dry weight basis.
IV - Percentage unfrozen water, green weight basis.

	June 21, 1932				July 21, 1932			
	I	II	III	IV	I	II	III	IV
Hibernal	70.0	14.4	33.8	10.1	65.2	16.9	31.7	11.0
Virginia	66.5	16.6	33.2	11.1	61.6	17.8	28.5	11.0
Shield's	66.4	16.6	32.8	11.0	62.0	18.9	30.8	11.7
Dudley	66.8	16.5	33.1	11.0	61.5	18.7	29.7	11.5
Okabena	69.6	13.6	31.3	9.5	66.1	16.4	32.0	10.8
Wealthy	68.3	15.0	32.3	10.2	63.5	17.0	29.4	11.8
Ioensis	69.3	15.0	33.9	10.4	64.0	17.1	30.2	10.9
Wolf River	66.3	15.1	29.9	10.1	63.8	16.8	29.6	10.7
Cortland	68.6	15.1	33.0	10.3	62.2	17.2	28.3	10.7
Baltimore	65.7	15.5	29.7	10.2	62.7	16.3	27.5	10.2
Jonathan	67.8	16.1	34.0	11.0	65.0	16.4	30.2	10.6
Delicious	69.4	14.0	31.6	9.7	66.4	14.9	29.4	9.9
Grimes	65.9	16.0	30.8	10.5	62.4	17.0	28.0	10.6
Wagner	66.6	16.1	32.1	10.7	62.1	17.9	29.5	11.1
Stayman	67.2	15.4	31.5	10.3	63.3	16.7	28.7	10.6

	August 20, 1932				September 21, 1932			
	I	II	III	IV	I	II	III	IV
Hibernal	66.3	19.7	38.8	13.1	57.5	25.3	34.2	14.5
Virginia	62.4	20.1	36.9	12.6	57.9	24.2	33.8	14.0
Shield's	63.1	20.4	37.9	12.9	58.4	23.2	32.8	13.6
Dudley	62.4	22.0	36.5	13.7	58.4	23.4	32.8	13.6
Okabena	65.2	21.4	40.1	13.9	59.2	22.5	32.7	13.3
Wealthy	61.3	20.7	32.8	12.7	58.2	22.1	30.9	12.9
Ioensis	63.0	23.7	38.1	14.9	54.6	28.8	34.6	15.7
Wolf River	68.6	18.8	37.5	12.9	61.4	20.8	33.2	12.8
Cortland	64.6	21.4	38.3	13.8	60.5	22.2	34.0	13.4
Baltimore	67.8	19.3	38.2	13.1	57.0	24.8	32.9	14.1
Jonathan	67.8	18.2	36.0	12.4	62.2	19.9	32.7	12.4
Delicious	64.4	24.5	44.2	15.7	59.7	22.7	33.7	13.5
Grimes	66.6	20.4	34.8	13.6	60.0	22.2	33.4	13.3
Wagner	65.6	20.0	39.1	13.1	55.8	28.0	35.1	15.6
Stayman	64.2	20.0	35.8	12.8	55.7	26.8	33.5	14.9

I - Percentage of water.
II - Percentage of water unfrozen.
III - Percentage unfrozen water, dry weight basis.
IV - Percentage unfrozen water, green weight basis.

	October 22, 1932				November 21, 1932			
	I	II	III	IV	I	II	III	IV
Hibernal	47.7	40.3	36.9	19.3	46.2	46.1	39.6	21.3
Virginia	46.8	38.8	34.3	18.2	47.5	44.4	40.2	21.1
Shield's	51.0	34.0	35.5	17.3	51.4	38.6	40.8	19.8
Dudley	49.3	39.1	38.0	19.3	47.5	44.7	40.5	21.2
Okabena	47.3	40.1	36.0	19.0	48.7	42.4	40.3	20.6
Wealthy	46.7	41.9	36.6	19.5	48.8	42.4	40.4	20.7
Ioensis	47.7	40.4	37.0	19.3	49.2	42.3	41.1	20.8
Wolf River	47.4	39.9	35.9	18.9	47.1	44.0	39.1	20.7
Cortland	47.4	41.0	37.0	19.4	49.3	41.0	40.0	20.2
Baltimore	49.8	36.5	36.2	18.1	46.7	44.6	39.2	20.8
Jonathan	47.2	40.0	35.7	18.8	47.7	42.5	38.8	20.3
Delicious	48.8	40.5	37.7	19.5	49.2	41.5	40.2	20.4
Grimes	48.5	37.1	35.0	18.0	47.5	43.1	39.0	20.5
Wagner	50.3	39.2	39.6	19.7	47.6	44.0	40.1	20.9
Stayman	45.5	42.9	35.7	19.4	47.5	44.0	39.8	20.9

	December 22, 1932				January 21, 1933			
	I	II	III	IV	I	II	III	IV
Hibernal	46.4	48.5	42.0	22.5	47.1	48.2	43.0	22.7
Virginia	47.8	45.4	41.5	21.7	48.6	46.0	43.6	22.4
Shield's	49.7	43.6	43.2	21.7	47.9	48.0	44.1	23.0
Dudley	48.6	45.4	43.0	22.1	49.5	44.4	43.6	22.0
Okabena	46.8	47.7	42.0	22.3	45.3	49.2	40.6	22.2
Wealthy	51.0	42.5	44.1	21.6	47.6	48.0	43.7	22.9
Ioensis	47.9	44.5	40.9	21.3	51.7	44.0	47.0	22.7
Wolf River	47.7	46.3	42.1	22.0	48.7	46.5	44.2	22.6
Cortland	48.6	45.6	43.2	22.2	47.3	47.5	42.7	22.5
Baltimore	49.3	42.9	41.7	21.2	48.7	45.1	43.9	22.0
Jonathan	47.7	45.9	41.8	21.9	46.5	50.4	43.7	23.4
Delicious	47.2	47.1	42.2	22.2	48.8	46.7	44.6	22.9
Grimes	46.9	46.3	41.0	21.7	44.7	51.8	41.8	23.1
Wagner	49.2	44.8	43.4	22.0	47.7	46.4	42.3	22.1
Stayman	48.7	43.8	41.5	21.3	48.3	47.6	44.5	23.0

I - Percentage of water.
II - Percentage of water unfrozen.
III- Percentage unfrozen water, dry weight basis.
IV - Percentage unfrozen water, green weight basis.

February 21, 1933

	I	II	III	IV
Hibernal	45.8	48.8	41.3	22.4
Virginia	47.2	47.6	42.7	22.5
Shield's	48.6	44.9	42.5	21.8
Dudley	47.6	47.7	43.5	22.7
Okabena	48.3	45.0	42.0	21.7
Wealthy	48.9	45.1	43.2	22.0
Ioensis	48.0	47.5	43.7	22.7
Wolf River	46.1	49.6	42.4	22.8
Cortland	48.3	46.6	43.6	22.5
Baltimore	47.0	47.7	42.4	22.5
Jonathan	46.1	49.7	42.6	23.0
Delicious	48.2	45.2	42.0	21.7
Grimes	46.1	49.5	42.4	22.8
Wagner	47.3	47.5	42.7	22.5
Stayman	46.5	47.5	41.4	22.1

March 24, 1933

	I	II	III	IV
Hibernal	46.8	50.4	44.6	23.6
Virginia	50.9	44.0	45.6	22.4
Shield's	51.3	43.2	45.5	22.1
Dudley	46.1	51.8	44.3	23.8
Okabena	48.6	46.7	44.2	22.7
Wealthy	49.7	46.1	45.6	23.0
Ioensis	48.6	47.0	44.6	22.9
Wolf River	48.2	48.5	45.1	23.3
Cortland	49.9	47.9	47.8	24.0
Baltimore	47.8	46.4	42.6	22.2
Jonathan	50.4	46.2	47.0	23.3
Delicious	50.4	44.6	45.4	22.5
Grimes	46.6	50.4	44.1	23.5
Wagner	49.5	45.8	45.0	22.7
Stayman	47.9	48.5	44.6	23.2

April 21, 1933

	I	II	III	IV
Hibernal	47.2	39.1	35.0	18.5
Virginia	48.2	36.3	33.8	17.5
Shield's	49.6	34.6	34.0	17.1
Dudley	46.7	39.9	35.0	18.6
Okabena	47.5	38.6	35.1	18.4
Wealthy	48.2	37.8	35.3	18.2
Ioensis	49.2	37.3	36.2	18.3
Wolf River	45.9	41.6	35.4	19.1
Cortland	47.3	36.6	34.7	18.2
Baltimore	47.7	36.3	33.2	17.3
Jonathan	48.1	36.3	33.6	17.4
Delicious	49.1	34.8	33.6	17.1
Grimes	46.2	38.2	32.8	17.7
Wagner	48.3	37.8	35.4	18.3
Stayman	47.1	38.2	34.0	18.0

May 20, 1933

	I	II	III	IV
Hibernal	58.1	24.2	33.6	14.1
Virginia	58.8	24.4	34.8	14.3
Shield's	58.2	24.2	33.8	14.1
Dudley	53.0	30.0	35.9	16.0
Okabena	55.2	26.6	32.8	14.7
Wealthy	55.4	26.0	32.2	14.4
Ioensis	56.0	28.2	36.0	15.8
Wolf River	55.5	28.3	35.5	15.7
Cortland	54.0	27.7	32.4	14.9
Baltimore	55.1	28.5	35.0	15.7
Jonathan	55.7	26.1	33.0	14.5
Delicious	55.2	27.5	33.9	15.2
Grimes	55.1	26.3	32.2	14.5
Wagner	53.8	30.6	35.7	16.5
Stayman	53.6	30.0	34.5	16.0

I - Percentage of water.
II - Percentage of water unfrozen.
III - Percentage unfrozen water, dry weight basis.
IV - Percentage unfrozen water, green weight basis.

(VII)

Water Relations in Tree Varieties When Frozen at - 20 C.

	July 21, 1932				August 20, 1932			
	I	II	III	IV	I	II	III	IV
Virginia	62.2	16.4	27.0	9.1	62.0	27.6	45.2	17.1
Wealthy	58.1	19.7	27.3	11.4	63.8	25.4	44.9	16.2
Wolf River	59.8	17.7	26.4	10.6	64.0	24.2	43.0	15.5
Cortland	59.1	18.3	26.4	10.8	62.1	24.2	39.7	15.0
Baltimore	59.2	18.7	27.1	11.0	59.0	30.1	43.3	17.7
Jonathan	57.2	19.1	25.5	10.9	59.8	26.2	35.7	15.7
Delicious	57.3	20.1	27.0	11.5	59.6	23.0	34.0	13.7
Grimes	56.4	20.1	26.0	11.3	61.2	23.7	37.4	14.5
Wagner	60.1	18.7	28.2	11.2	64.7	21.1	38.6	13.6
Stayman	57.8	19.6	26.9	11.4	56.3	27.5	35.5	15.5

	September 21, 1932				October 22, 1932			
	I	II	III	IV	I	II	III	IV
Virginia	53.6	27.7	32.0	14.8	48.5	35.6	33.6	17.5
Wealthy	52.0	29.5	32.0	15.4	46.5	39.1	34.0	18.2
Wolf River	55.3	26.0	32.1	14.3	49.8	34.7	34.5	17.3
Cortland	55.2	26.1	32.1	14.3	46.8	38.4	33.8	18.0
Baltimore	53.4	27.5	31.6	14.7	45.9	39.1	33.2	18.0
Jonathan	52.1	27.2	29.6	14.1	46.3	35.3	30.5	16.3
Delicious	52.3	29.0	31.8	15.2	46.5	35.7	31.1	16.6
Grimes	55.3	24.3	30.1	13.5	48.0	34.4	31.8	16.5
Wagner	53.6	28.6	33.0	15.3	47.8	35.8	32.8	17.1
Stayman	52.0	29.3	31.6	15.2	45.1	38.0	31.3	17.1

	November 21, 1932				December 22, 1932			
	I	II	III	IV	I	II	III	IV
Virginia	48.9	40.4	38.7	19.8	48.5	41.0	38.7	19.9
Wealthy	46.6	43.8	38.2	20.4	47.0	43.8	38.8	20.6
Wolf River	48.7	40.0	38.1	19.5	48.5	41.0	38.6	19.8
Cortland	47.8	41.7	38.2	20.0	47.8	41.9	38.4	20.0
Baltimore	46.4	41.7	36.1	19.3	45.5	42.7	35.8	19.4
Jonathan	47.0	41.2	36.6	19.4	47.1	41.1	36.6	19.3
Delicious	47.4	40.0	36.1	19.0	46.8	42.6	37.6	20.0
Grimes	48.0	40.2	37.0	19.2	47.0	42.6	37.9	20.0
Wagner	47.8	39.8	36.5	19.0	48.2	41.2	38.7	20.0
Stayman	45.8	43.0	36.3	19.7	45.6	42.7	35.8	19.5

I - Percentage of water.
II - Percentage of water unfrozen.
III - Percentage unfrozen water, dry weight basis.
IV Percentage unfrozen water, green weight basis.

(VIII)

	January 21, 1933				February 21, 1933			
	I	II	III	IV	I	II	III	IV
Virginia	48.7	45.5	43.3	22.2	46.8	43.5	38.3	20.3
Wealthy	47.2	48.1	43.0	22.7	45.8	46.6	39.4	21.3
Wolf River	47.0	48.1	42.8	22.6	46.4	45.3	39.3	21.0
Cortland	47.5	48.5	44.0	23.0	46.7	44.7	39.1	20.8
Baltimore	45.5	50.8	42.5	23.1	44.7	47.0	38.0	21.0
Jonathan	49.1	46.6	45.0	22.8	46.0	46.2	39.4	21.2
Delicious	47.9	45.8	42.2	22.0	46.5	43.3	37.8	20.2
Grimes	48.7	44.9	42.7	21.9	47.0	43.2	38.2	20.3
Wagner	47.9	43.9	40.4	21.0	46.4	43.3	37.7	20.1
Stayman	47.0	47.8	42.4	22.4	45.7	44.3	37.3	20.2

	March 24, 1933				April 21, 1933			
	I	II	III	IV	I	II	III	IV
Virginia	47.9	41.0	37.8	19.6	47.9	37.3	34.3	17.8
Wealthy	46.1	45.2	38.7	20.8	46.6	38.1	33.3	17.7
Wolf River	46.4	44.8	38.8	20.8	45.6	41.9	35.3	19.2
Cortland	47.7	41.8	38.1	20.0	48.1	39.1	36.2	18.8
Baltimore	47.4	41.9	37.7	19.8	47.4	37.3	33.5	17.6
Jonathan	46.7	42.2	37.0	19.7	48.7	33.1	31.3	16.1
Delicious	47.2	43.0	38.6	20.3	48.7	34.4	32.7	16.7
Grimes	46.8	42.6	37.5	20.0	48.5	35.2	33.1	17.0
Wagner	48.2	30.8	38.0	19.6	48.6	34.2	32.4	16.6
Stayman	47.2	40.9	36.6	19.5	47.3	36.3	32.6	17.2

	May 20, 1933			
	I	II	III	IV
Virginia	57.1	23.4	31.1	13.3
Wealthy	55.5	23.7	29.6	13.1
Wolf River	57.6	22.1	30.1	12.7
Cortland	56.2	23.6	30.2	13.2
Baltimore	51.0	30.8	32.1	15.7
Jonathan	51.8	19.6	25.7	11.1
Delicious	55.0	25.6	31.2	14.0
Grimes	55.6	24.6	31.0	13.7
Wagner	56.5	22.6	29.5	12.8
Stayman	52.7	28.0	31.1	14.7

I - Percentage of water.
II - Percentage of water unfrozen.
III - Percentage unfrozen water, dry weight basis.
IV - Percentage unfrozen water, green weight basis.

Water Relations in Varieties When Heated One Hour at 80° C., Frozen at - 20° C.

	July 21, 1931			August 22, 1931		
	I	II	IV	I	II	IV
Hibernal	66.1	21.5	14.1	63.0	26.3	16.6
Virginia	60.9	22.9	13.9	57.4	31.6	18.1
Shield's	60.4	25.5	15.3	-- -	-- -	-- -
Dudley	60.4	24.8	14.9	57.6	26.2	15.1
Okabena	63.8	20.0	12.7	62.2	28.5	17.7
Wealthy	58.0	26.4	15.3	56.3	23.4	13.2
Ioensis	63.2	22.7	14.3	59.3	30.2	17.9
Wolf River	65.5	21.0	13.7	63.1	25.2	15.9
Cortland	63.6	20.9	13.3	65.2	24.5	16.0
Baltimore	61.2	23.2	14.8	58.6	25.8	15.1
Jonathan	60.5	25.5	15.4	58.1	27.8	16.2
Delicious	64.4	21.9	14.0	63.3	22.0	13.9
Grimes	60.0	25.1	15.0	59.9	29.9	17.9
Wagner	62.3	22.3	13.9	57.9	31.1	18.0
Stayman	60.1	28.3	17.0	59.6	32.7	19.5

	September 21, 1931			October 21, 1931		
	I	II	IV	I	II	IV
Hibernal	56.2	31.8	17.9	50.9	35.0	17.8
Virginia	58.2	35.9	20.9	52.3	34.9	18.2
Shield's	59.8	29.9	17.9	54.1	34.0	18.4
Dudley	-- -	-- -	-- -	52.5	34.4	18.0
Okabena	61.7	28.3	17.4	-- -	-- -	-- -
Wealthy	54.8	34.5	18.9	52.3	35.7	18.7
Ioensis	59.1	29.3	16.7	51.0	38.0	19.4
Wolf River	62.5	26.3	16.4	-- -	-- -	-- -
Cortland	63.5	23.6	15.0	56.4	29.1	16.5
Baltimore	-- -	-- -	-- -	51.6	32.9	17.0
Jonathan	58.3	16.4	9.6	53.2	30.8	16.2
Delicious	61.0	22.0	13.4	55.7	28.2	15.7
Grimes	61.5	29.0	17.8	55.5	28.1	15.6
Wagner	-- -	-- -	-- -	54.2	28.8	15.3
Stayman	-- -	-- -	-- -	50.3	35.9	18.0

I - Percentage of water.
II - Percentage of water unfrozen.
IV - Percentage unfrozen water, green weight basis.

	November 21, 1931			December 21, 1931		
	I	II	IV	I	II	IV
Hibernal	49.2	41.4	20.3	49.5	40.4	20.0
Virginia	51.1	38.5	19.6	49.4	41.4	20.4
Shield's	52.1	38.5	20.0	51.5	39.0	20.1
Dudley	49.0	41.2	20.2	48.4	44.0	21.3
Okabena	51.2	38.3	19.6	49.1	42.0	20.6
Wealthy	52.2	35.2	18.4	49.4	40.8	20.1
Ioensis	48.8	40.3	19.6	50.3	41.6	20.9
Wolf River	51.8	35.5	18.4	51.0	37.5	19.1
Cortland	50.7	36.8	18.6	50.6	37.5	19.0
Baltimore	49.4	39.1	19.3	49.6	38.7	19.2
Jonathan	49.1	38.1	18.7	50.5	36.6	18.5
Delicious	51.1	35.5	18.2	50.0	39.7	19.9
Grimes	47.1	40.1	18.9	49.0	40.1	19.6
Wagner	49.6	40.2	19.9	48.6	39.5	19.2
Stayman	50.1	37.2	18.6	49.2	39.0	19.2

	January 23, 1932			February 20, 1932		
	I	II	IV	I	II	IV
Hibernal	48.7	42.1	20.5	46.7	47.1	22.0
Virginia	50.8	40.9	20.7	47.1	31.4	14.8
Shield's	51.6	41.3	21.3	50.6	41.4	21.0
Dudley	50.6	40.0	20.2	48.9	43.2	21.1
Okabena	47.9	42.0	20.0	48.7	41.2	20.0
Wealthy	49.5	40.7	20.1	49.2	42.4	20.8
Ioensis	49.7	43.2	21.5	48.4	43.2	20.9
Wolf River	51.1	38.0	19.4	51.3	39.0	20.0
Cortland	49.4	40.9	20.2	50.0	39.1	19.5
Baltimore	48.4	40.3	19.5	49.7	39.6	19.7
Jonathan	50.0	40.0	20.0	48.8	39.5	19.3
Delicious	52.3	36.5	19.0	49.0	40.1	19.7
Grimes	47.0	41.7	19.6	45.4	46.4	21.1
Wagner	47.5	42.3	20.1	46.7	43.5	20.3
Stayman	-- -	-- -	-- -	-- -	-- -	-- -

I - Percentage of water.
II - percentage of water unfrozen.
IV - Percentage unfrozen water, green weight basis.

	March 21, 1932			April 21, 1932		
	I	II	IV	I	II	IV
Hibernal	45.3	48.6	22.0	50.7	37.6	19.0
Virginia	48.5	44.2	21.4	52.6	35.0	18.4
Shield's	50.9	40.4	20.5	55.3	32.4	17.9
Dudley	49.5	44.0	21.8	51.4	37.0	19.0
Okabena	49.9	40.3	20.0	49.4	41.4	20.5
Wealthy	48.4	40.8	19.8	49.8	38.5	19.2
Ioensis	48.2	44.4	21.4	50.7	43.0	21.8
Wolf River	49.8	40.5	20.1	49.6	38.5	19.1
Cortland	-- -	-- -	-- -	-- -	-- -	-- -
Baltimore	48.0	43.6	21.0	50.1	38.9	19.5
Jonathan	48.5	43.7	21.2	53.3	29.3	15.6
Delicious	49.8	39.5	19.6	52.6	35.0	18.4
Grimes	47.5	44.9	21.3	49.2	38.3	18.9
Wagner	-- -	-- -	-- -	50.0	36.7	18.4
Stayman	-- -	-- -	-- -	-- -	-- -	-- -

	May 21, 1932		
	I	II	IV
Hibernal	61.5	24.5	15.1
Virginia	60.9	24.7	15.0
Shield's	61.2	26.7	16.4
Dudley	57.0	27.5	15.7
Okabena	62.0	23.6	14.7
Wealthy	59.2	26.4	15.7
Ioensis	58.5	30.5	17.8
Wolf River	59.9	25.3	15.1
Cortland	60.3	24.1	14.5
Baltimore	59.8	22.9	13.7
Jonathan	59.4	22.5	14.4
Delicious	59.6	24.3	14.5
Grimes	57.5	29.0	16.7
Wagner	57.9	26.1	15.1
Stayman	59.8	24.8	14.8

I - Percentage of water.
II - Percentage of water unfrozen.
IV - Percentage unfrozen water, green weight basis.

Water Relations in Varieties When Frozen at - 5°C.

	July 21, 1932			August 20, 1932
	I	II	IV	
Hibernal	64.8	38.8	25.1	No determination at -5° on this date.
Virginia	61.6	37.3	23.0	
Shield's	62.0	37.1	23.0	
Dudley	61.6	36.7	22.6	
Okabena	66.2	30.1	19.9	
Wealthy	62.7	38.4	24.0	
Ioensis	63.0	35.8	22.6	
Wolf River	63.8	34.7	22.1	
Cortland	63.9	36.4	23.3	
Baltimore	62.8	35.5	22.3	
Jonathan	65.0	34.8	22.6	
Delicious	66.0	34.7	22.8	
Grimes	61.8	39.4	24.3	
Wagner	59.6	42.9	25.5	
Stayman	63.6	36.2	23.0	

	September 21, 1932			October 22, 1932		
	I	II	IV	I	II	IV
Hibernal	57.5	44.3	25.5	48.0	52.4	25.2
Virginia	58.4	42.0	24.5	46.9	52.5	24.6
Shield's	58.9	41.4	24.4	50.2	50.4	25.3
Dudley	57.8	42.1	24.3	48.6	54.3	26.3
Okabena	58.8	40.4	23.7	46.7	54.7	25.5
Wealthy	59.4	42.3	25.1	46.4	58.2	27.0
Ioensis	54.4	46.6	25.3	47.8	55.4	26.4
Wolf River	61.6	39.5	24.3	46.7	56.0	26.1
Cortland	60.5	44.2	26.7	46.8	56.4	26.4
Baltimore	57.3	43.7	25.0	48.9	57.2	27.9
Jonathan	62.0	39.7	24.6	47.2	59.6	28.1
Delicious	59.0	43.3	25.6	48.3	55.7	26.9
Grimes	61.0	40.8	24.8	48.4	55.4	26.7
Wagner	55.1	43.5	26.7	50.3	57.7	29.0
Stayman	55.4	44.3	24.5	46.0	59.7	27.5

I - Percentage of water.
II - Percentage of water unfrozen.
IV - Percentage unfrozen water, green weight basis.

	November 21, 1932			December 22, 1932		
	I	II	IV	I	II	IV
Hibernal	46.1	56.5	26.0	45.9	58.9	27.0
Virginia	47.9	53.8	25.8	47.3	55.4	26.2
Shield's	51.7	47.9	24.8	49.8	52.5	26.1
Dudley	47.6	57.2	27.2	47.7	57.4	27.4
Okabena	48.4	54.1	26.2	47.1	57.8	27.2
Wealthy	48.6	55.2	26.8	49.9	53.7	26.8
Ioensis	49.4	53.2	26.3	47.6	55.0	26.2
Wolf River	46.4	57.7	26.8	47.9	56.8	27.2
Cortland	49.0	55.7	27.3	48.4	57.6	27.9
Baltimore	46.4	57.7	26.8	48.7	58.1	28.2
Jonathan	47.8	57.9	27.7	47.6	60.2	28.6
Delicious	49.2	54.7	26.9	46.7	60.2	28.0
Grimes	48.0	57.3	27.5	46.9	58.8	27.5
Wagner	47.4	56.0	26.6	49.1	55.6	27.2
Stayman	47.2	59.3	28.0	48.1	59.1	28.4

	January 21, 1933			February 21, 1933		
	I	II	IV	I	II	IV
Hibernal	46.7	62.6	29.2	46.0	61.8	28.4
Virginia	47.8	58.8	28.1	47.4	60.3	28.6
Shield's	47.4	60.0	28.4	48.5	56.9	27.6
Dudley	49.4	56.2	27.7	47.2	58.8	27.7
Okabena	44.9	60.3	27.0	47.9	56.8	27.2
Wealthy	47.1	60.6	28.5	48.6	58.0	28.2
Ioensis	51.6	53.8	27.8	48.1	58.4	28.1
Wolf River	48.0	57.8	27.8	46.0	64.5	29.6
Cortland	46.2	62.5	28.9	48.6	60.2	29.3
Baltimore	48.0	60.9	29.2	46.8	60.6	28.3
Jonathan	45.9	65.8	30.2	45.7	66.6	30.5
Delicious	48.2	58.8	28.3	47.6	60.0	28.6
Grimes	44.2	66.1	29.3	45.4	65.6	29.8
Wagner	46.9	60.7	28.5	47.5	59.9	28.4
Stayman	47.5	63.7	30.3	46.6	64.1	29.9

I - Percentage of water.
II - Percentage of water unfrozen.
IV - Percentage unfrozen water, green weight basis.

	March 24, 1933			April 21, 1933		
	I	II	IV	I	II	IV
Hibernal	46.0	60.9	28.0	47.2	53.2	25.1
Virginia	49.6	51.5	25.6	48.2	49.4	23.8
Shield's	50.1	49.8	24.9	49.6	47.7	23.7
Dudley	45.6	61.2	27.9	46.7	54.9	25.6
Okabena	47.5	57.2	27.2	47.5	49.9	23.7
Wealthy	48.6	56.0	27.2	48.2	51.6	24.8
Ioensis	48.3	55.5	26.8	49.2	48.6	23.9
Wolf River	47.3	59.2	28.0	45.9	55.3	25.4
Cortland	49.1	60.9	29.9	47.3	51.3	24.3
Baltimore	47.5	57.9	27.5	47.7	51.1	24.4
Jonathan	49.3	59.2	29.2	48.1	52.7	25.4
Delicious	49.2	56.6	27.9	49.1	50.4	24.8
Grimes	45.0	63.3	28.5	46.2	54.5	25.2
Wagner	48.0	56.8	27.2	48.3	51.4	24.8
Stayman	47.0	60.2	28.3	47.1	55.3	26.1

	May 20, 1933		
	I	II	IV
Hibernal	58.6	43.1	25.3
Virginia	59.0	41.7	24.6
Shield's	59.8	40.1	23.9
Dudley	53.0	43.3	22.9
Okabena	55.7	41.4	23.0
Wealthy	55.8	47.0	26.2
Ioensis	56.3	43.1	24.3
Wolf River	56.3	44.2	24.8
Cortland	54.9	46.7	25.6
Baltimore	54.9	43.3	23.7
Jonathan	56.7	45.6	25.9
Delicious	55.9	46.2	25.8
Grimes	55.5	44.6	24.8
Wagner	55.0	48.4	26.6
Stayman	53.3	45.6	24.3

I - Percentage of water.
II - Percentage of water unfrozen.
IV - Percentage unfrozen water, green weight basis.